THE
BIOLOGICAL BULLETIN

PUBLISHED BY
THE MARINE BIOLOGICAL LABORATORY

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THE BIOLOGICAL BULLETIN

PUBLISHED BY THE MARINE BIOLOGICAL LABORATORY

THE MARINE BIOLOGICAL LABORATORY

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I. TRUSTEES

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CHARLES PACKARD, *Director*, Marine Biological Laboratory
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Albert Tyler, California Institute of Technology

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P. S. Galtsoff, U. S. Fish and Wild Life Service
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A. H. Sturtevant, California Institute of Technology

TO SERVE UNTIL 1950

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D. P. Costello, University of North Carolina
M. H. Jacobs, University of Pennsylvania
D. A. Marsland, New York University
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Charles Packard, *Ex officio*

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Ralph Wichterman

II. ACT OF INCORPORATION

No. 3170

COMMONWEALTH OF MASSACHUSETTS

Be It Known, That whereas Alpheus Hyatt, William Sanford Stevens, William T. Sedgwick, Edward G. Gardiner, Susan Minns, Charles Sedgwick Minot, Samuel Wells, William G. Farlow, Anna D. Phillips, and B. H. Van Vleck have associated themselves with the intention of forming a Corporation under the name of the Marine Biological Laboratory, for the purpose of establishing and maintaining a laboratory or station for scientific study and investigation, and a school for instruction in biology and natural history, and have complied with the provisions of the statutes of this Commonwealth in such case made and provided, as appears from the certificate of the President, Treasurer, and Trustees of said Corporation, duly approved by the Commissioner of Corporations, and recorded in this office;

Now, therefore, I, HENRY B. PIERCE, Secretary of the Commonwealth of Massachusetts, *do hereby certify* that said A. Hyatt, W. S. Stevens, W. T. Sedgwick, E. G. Gardiner, S. Minns, C. S. Minot, S. Wells, W. G. Farlow, A. D. Phillips, and B. H. Van Vleck, their associates and successors, are legally organized and established as, and are hereby made, an existing Corporation, under the name of the MARINE BIOLOGICAL LABORATORY, with the powers, rights, and privileges, and subject to the limitations, duties, and restrictions, which by law appertain thereto.

Witness my official signature hereunto subscribed, and the seal of the Commonwealth of Massachusetts hereunto affixed, this twentieth day of March, in the year of our Lord One Thousand Eight Hundred and Eighty-Eight.

[SEAL]

HENRY B. PIERCE,
Secretary of the Commonwealth.

III. BY-LAWS OF THE CORPORATION OF THE MARINE BIOLOGICAL LABORATORY

I. The members of the Corporation shall consist of persons elected by the Board of Trustees.

II. The officers of the Corporation shall consist of a President, Vice President, Director, Treasurer, and Clerk.

III. The Annual Meeting of the members shall be held on the second Tuesday in August in each year, at the Laboratory in Woods Hole, Massachusetts, at 11:30 A.M., and at such meeting the members shall choose by ballot a Treasurer and a Clerk to serve one year, and eight Trustees to serve four years, and shall transact such other business as may properly come before the meeting. Special meetings of the members may be called by the Trustees to be held at such time and place as may be designated.

IV. Twenty-five members shall constitute a quorum at any meeting.

V. Any member in good standing may vote at any meeting, either in person or by proxy duly executed.

VI. Inasmuch as the time and place of the Annual Meeting of members are fixed by these By-laws, no notice of the Annual Meeting need be given. Notice of any special meeting of members, however, shall be given by the Clerk by mailing notice of the time

and place and purpose of such meeting, at least fifteen (15) days before such meeting, to each member at his or her address as shown on the records of the Corporation.

VII. The Annual Meeting of the Trustees shall be held on the second Tuesday in August in each year, at the Laboratory in Woods Hole, Mass., at 10 A.M. Special meetings of the Trustees shall be called by the President, or by any seven Trustees, to be held at such time and place as may be designated, and the Secretary shall give notice thereof by written or printed notice, mailed to each Trustee at his address as shown on the records of the Corporation, at least one (1) week before the meeting. At such special meeting only matters stated in the notice shall be considered. Seven Trustees of those eligible to vote shall constitute a quorum for the transaction of business at any meeting.

VIII. There shall be three groups of Trustees:

(A) Thirty-two Trustees chosen by the Corporation, divided into four classes, each to serve four years; and in addition there shall be two groups of Trustees as follows:

(B) Trustees ex officio, who shall be the President and Vice President of the Corporation, the Director of the Laboratory, the Associate Director, the Treasurer, and the Clerk;

(C) Trustees Emeriti, who shall be elected from *present* or *former* Trustees by the Corporation. Any regular Trustee who has attained the age of seventy years shall continue to serve as Trustee until the next Annual Meeting of the Corporation, whereupon his office as regular Trustee shall become vacant and be filled by election by the Corporation and he shall become eligible for election as Trustee *Emeritus* for life. The Trustees *ex officio* and *Emeritus* shall have all the rights of the Trustees except that Trustees *Emeritus* shall not have the right to vote.

The Trustees and officers shall hold their respective offices until their successors are chosen and have qualified in their stead.

IX. The Trustees shall have the control and management of the affairs of the Corporation; they shall elect a President of the Corporation who shall also be Chairman of the Board of Trustees; and shall also elect a Vice President of the Corporation who shall also be the Vice Chairman of the Board of Trustees; they shall appoint a Director of the Laboratory; and they may choose such other officers and agents as they may think best; they may fix the compensation and define the duties of all the officers and agents; and may remove them, or any of them, except those chosen by the members, at any time; they may fill vacancies occurring in any manner in their own number or in any of the offices. The Board of Trustees shall have the power to choose an Executive Committee from their own number, and to delegate to such Committee such of their own powers as they may deem expedient. They shall from time to time elect members to the Corporation upon such terms and conditions as they may think best.

X. Any person interested in the Laboratory may be elected by the Trustees to a group to be known as Associates of the Marine Biological Laboratory.

XI. The consent of every Trustee shall be necessary to dissolution of the Marine Biological Laboratory. In case of dissolution, the property shall be disposed of in such manner and upon such terms as shall be determined by the affirmative vote of two-thirds of the Board of Trustees.

XII. The account of the Treasurer shall be audited annually by a certified public accountant.

XIII. These By-laws may be altered at any meeting of the Trustees, provided that the notice of such meeting shall state that an alteration of the By-laws will be acted upon.

IV. REPORT OF THE TREASURER

To the Trustees of the Marine Biological Laboratory:

Gentlemen:

The year 1949 was notable from the financial viewpoint because of: 1) the receipt from the Rockefeller Foundation of $150,000 for the restoration and improvement of the "Old Main" laboratory, $25,000 for current expenses, and $4,905.44 in equipment; 2) the gift of a power cruiser, renamed the "Arbacia," from Mrs. John G. Ralston; and 3) the inauguration of the "Frank R. Lillie Memorial Fund" under the able leadership of Dr. G. H. A. Clowes.

As a result of the Rockefeller and other gifts for capital items, the total assets of the Laboratory increased during the year by $173,021.65 to a total of $2,711,621.96.

The current operations for 1949, according to the report of the auditors, Seamans, Stetson and Tuttle of Boston, show total cash receipts of $307,635.14 and disbursements of $310,100.93, the latter including $281,372.89 for current expenses and $28,728.04 for "additions to capital assets" (equipment, etc.). Current income cash on hand December 31, 1949, amounted to $10,290.80. The totals of receipts and disbursements for 1949 were about the same as those for 1948, and this was true for most of the sub-totals for the various departments. One notable exception was the Supply Department where total sales declined from $94,701.77 to $78,770.38. Actual cash receipts in 1949 for the Supply Department were $82,663.36 and expenditures were $82,494.33 showing a net profit of $169.03. These cash figures, however, do not take into account credits from the Instruction and Research Departments for supplies furnished, differences of inventories, depreciation, etc. The complete statement of the auditors shows net income for the Supply Department of $12,573.29.

The Balance Sheet, Statement of Current Surplus, and Summary of Cash Transactions, as given in the auditors' report, follow:

Marine Biological Laboratory Balance Sheet, Dec. 31, 1949

Assets

Endowment Assets and Equities:			
Securities and Cash in Hands of Central Hanover Bank and Trust Company, New York, Trustee		$ 966,928.06	
Securities and Cash in Minor Funds		18,465.54	$ 985,393.60
Plant Assets:			
Land	$ 133,626.38		
Buildings	1,357,661.51		
Equipment	256,825.70		
Library	390,133.36		
	$2,118,246.95		
Less Reserve for Depreciation	774,840.83	$1,343,406.12	
Book Fund, Securities and Cash		5,961.99	$1,349,368.11

Current Assets:

Cash		$ 16,620.21	
Mortgage Note Receivable		2,350.00	
Accounts Receivable		25,111.96	
Inventories:			
Supply Department	$ 52,482.99		
"Biological Bulletin"	16,004.65	$ 68,487.64	
Investments:			
Devil's Lane Property	$ 38,826.68		
Stock in General Biological Supply House	12,700.00		
Other Investment Securities	36,020.00		
Retirement Fund	20,557.08	$ 106,103.76	
Prepaid Insurance		6,665.25	
Items in Suspense (Debits)		1,521.43	
Unadjusted Debits:			
Old Main B'ldg Modernizing:			
Expenditures to Dec. 31	$ 119,279.36		
Cash Unexpended	30,720.64	$ 150,000.00	$ 376.860.25
			$2,711,621.96

Liabilities

Endowment Funds:

Endowment Funds	$ 965,433.05		
Reserve for Amortization	1,495.01	$ 966,928.06	
Minor Funds		18,465.54	$ 985,393.60
Plant Funds:			
Mortgage Notes Payable		$ 12,000.00	
Donations and Gifts	$1,181,564.04		
Other Investments in Plant from Gifts and Current Funds	155,804.07	$1,337,368.11	$1,349,368.11
Current Liabilities and Surplus:			
Accounts Payable		$ 8,850.56	
Items in Suspense (Credits)		2,566.09	
Current Surplus		215,443.60	
Grant of Rockefeller Foundation to Modernize Old Main Building		150,000.00	$ 376,860.25
			$2,711,621.96

Current Surplus Account

Balance, January 1, 1949			$ 209,432.17
Add:			
Excess of Income over Expense for Year		$ 11,499.89	
Reserve for Depreciation, 1949, Charged to Plant Funds		27,919.94	$ 39,419.83
			$ 248,852.00

Deduct:			
Payments from Current Funds during year for Plant Assets:			
Land	$ 3,000.00		
Buildings	11,704.65		
Equipment	11,030.39		
Library	9,673.36		
	$ 35,408.40		
Less Mortgage Note given in Partial Payment for Elliot Property	$ 6,000.00	$ 29,408.40	
Payment on Principal of Mortgage Note given in Partial Payment for "Dolphin"		4,000.00	$ 33,408.40
Balance, December 31, 1949			$ 215,443.60

Summary of Cash Transactions for Year ended Dec. 31, 1949

		Expenditures		
Current Cash	Receipts	Current	Additions to Capital Assets	Total Expenditures
Income from Endowment Funds	$ 40,347.15			
Income from Other Investments	22,159.95			
Real Estate Rentals	7,830.00	$ 2,877.40		$ 2,877.40
Donations	27,602.16			
Membership Dues	2,364.00			
Instruction	12,508.24	9,789.34		9,789.34
Research (including Apparatus and Chemical Departments)	27,127.63	26,091.04	$ 3,361.91	29,452.95
Supply Department	82,663.36	82,215.76	278.57	82,494.33
Mess	40,773.70	38,039.93		38,039.93
Dormitories	19,417.05	10,873.32	6,333.54	17,206.86
"Biological Bulletin"	6,568.78	9,389.46		9,389.46
Library	8,702.67	7,798.35	11,263.01	19,061.36
Book Purchase Account, Woods Hole Oceanographic Institution	1,200.00	1,137.62		1,137.62
Real Estate Sales (received on account)	5,007.00			
Miscellaneous	3,363.45			
Buildings and Grounds		41,558.69	3,491.01	45,049.70
Administration		29,367.71		29,367.71
Interest, Insurance, Cost of Living Bonus, and other Property Maintenance (not allocated)		14,537.82		14,537.82
Payment to Retirement Fund		7,696.45		7,696.45
Payment on Mortgage			4,000.00	4,000.00
	$307,635.14	$281,372.89	$28,728.04	$310,100.93
Cash Balance, January 1, 1949:				
Central Hanover Bank & Trust Co.		$ 5,487.16		
Falmouth National Bank		7,269.43	$ 12,756.59	
Receipts			307,635.14	
			$320,391.73	
Payments			310,100.93	
Cash Balance, December 31, 1949:				
Central Hanover Bank & Trust Co.		$ 8,159.11		
Falmouth National Bank		2,131.69	$ 10,290.80	

Other Cash Accounts	Balance Jan. 1, 1949	Received	Paid	Balance Dec. 31, 1949
Memhard Fund Cash	$ 33.05	$ 25.00		$ 58.05
Rev. Arsenious Boyer Burse Cash	436.53	125.00		561.53
Lucretia Crocker Fund Cash	1,510.36	357.52	$ 120.00	1,747.88
Bathing Beach (Clowes) Fund	1,000.00			1,000.00
Dr. Frank R. Lillie Memorial Fund Cash	1,000.62	3,951.85		4,952.47
Old Main Building Modernizing Fund Cash		150,000.00	119,279.36	30,720.64
Retirement Fund Cash	1,430.64		5,761.67	4,555.05
Income		1,189.63		
Transfer		7,696.45		
Bio Club Scholarship Fund	187.53	62.50	120.00	130.03
Book Fund	1,416.15		5,702.67	838.93
Income		123.87		
Sale of Securities		5,001.58		
Lalor Foundation Fellowships Cash	878.15	5,000.00	3,759.94	2,118.21

Respectfully submitted,
DONALD M. BRODIE,
Treasurer

V. REPORT OF THE LIBRARIAN

The sum of $11,164 was appropriated for the year 1949, plus $3000 from the Woods Hole Oceanographic Institution to be applied to staff salaries. The detailed account of the expenditure of the budget is covered in the Treasurer's report. The sum of $1200 was also appropriated by the Woods Hole Oceanographic Institution to cover library acquisitions.

During the year, 1353 (52 new) current journals were received. Of these 417 (11 new) were Marine Biological Laboratory subscriptions, 541 (13 new) were exchanges, and 154 (10 new) were gifts; 66 (2 new) were Woods Hole Oceanographic Institution subscriptions, 158 (13 new) were exchanges and 17 (3 new) were gifts. The noticeable increase in the number of current journals over that for 1948 is due to the fact that the German subscriptions have been resumed—56 out of 129 titles having been re-established since the war.

The Marine Biological Laboratory purchased 72 books, received 11 complimentary copies from authors, 46 gifts from the publishing firms, 37 from the E. L. Mark Library and 26 miscellaneous donations. The Woods Hole Oceanographic Institution purchased 28 titles. A total of 220 books was added to the shelves, the most outstanding purchase being Poggendorff's "Biographisch-literarisches Handwörterbuch" in ten volumes.

There were 9 back sets completed: 2 by purchase (Woods Hole Oceanographic Institution); 6 by exchange (3, Woods Hole Oceanographic Institution) and 1 by gift; 11 were partially completed: 3 by purchase (Woods Hole Oceanographic Institution); 7 by exchange (3, Woods Hole Oceanographic Institution) and 1 by gift.

The reprint additions to the Library numbered 8407. Of these, 1449 were of current issue and 6958 of earlier dates. Gifts from Drs. W. E. Garrey, Mary D. Rogick and Dorothy Wrinch totalled 1208 papers, 394 of which were not already in the Library's collection.

A sum of $5579.61 from the Carnegie Corporation of New York Fund was spent for 8 books, 12 completed back sets, 34 partially completed back sets, and the war-time accumulation of German serials.

Forty-six volumes were borrowed on inter-library loan, and 85 were sent to other institutions. Ninety-seven microfilm orders were filled to the amount of $322.24.

During the year, the sorting of the E. L. Mark Library was completed, and a final report is herewith given: 20,307 reprints were received, 6850 of which were added to the library's collection; 192 books, and 236 serial volumes and numbers. The duplicate material has been placed in the sales-room for clearance.

Grateful acknowledgment is made to Dr. A. W. Pappenheimer for the gift of his valuable collection of reprints. Mr. Henry Stommel's gift of 16 books is also worthy of an expression of appreciation.

The amount of $682.02 was realized from the sales-room. This sum was used to purchase a microfilm camera to replace the old one, which had depreciated to the point of uselessness. The new apparatus has been installed in a recently acquired photography room located next to the librarian's office.

At the end of the year 1949, the Library contained 58,800 bound volumes and 168,935 reprints.

In closing this report, the Librarian wishes to express her gratitude to the members of the Library Committee for their helpfulness throughout the year.

Respectfully submitted,

DEBORAH LAWRENCE,
Librarian

VI. REPORT OF THE DIRECTOR

TO THE TRUSTEES OF THE MARINE BIOLOGICAL LABORATORY:

Gentlemen:

I submit herewith a report of the sixty-second session of the Marine Biological Laboratory for the year 1949.

1. *Changes in Personnel*

At the Annual Meeting of the Trustees held August 6, 1949, and at the adjourned meeting of August 23rd, resignations were presented by the following:

Lawrason Riggs, President of the Corporation since 1942, and its Treasurer from 1924 to 1942. He stated that rotation in office is desirable, and that since he has served on the Executive Committee for 25 years it was time that he should be replaced.

E. Newton Harvey, Vice President from 1942. Dr. Harvey wished to be relieved of the duties incumbent on this office so that he might devote more time to research.

Charles Packard, Assistant Director and Director since 1937. Having reached the retirement age, he asked that a new Director be appointed in his place.

Frank M. MacNaught, Business Manager since 1913, and already past the age set for retirement, requested that he be retired. An appreciation of his long and devoted service to the Laboratory appears in the appendix of this report.

A Committee, of which Dr. Harvey was Chairman, was appointed to nominate new officers. At the December Meeting of the Trustees, Dr. Philip B. Armstrong was proposed as the new Director and was unanimously elected. The choice is most fortunate. Dr. Armstrong has played an important part in Laboratory affairs for many years, and is thoroughly familiar with the problems with which we are faced. He assumes his duties at a time when plans for developing the resources of the Laboratory are being considered. Under his direction they will be put into operation.

The Executive Committee elected Homer P. Smith as General Manager to replace Mr. MacNaught. He has already served as Assistant Business Manager for three and one-half years.

It is worth noting that within the years 1947–1950 the President, Vice President, Director, Librarian, Manager of the Apparatus Department, Business Manager, and the Superintendent of Buildings and Grounds have been or will be replaced. The terms of office ranged from 8 to 37 years, the average being nearly 20 years.

2. *Organization Problems*

During the summer a Committee, of which Dr. Ball was Chairman, made a thorough study of the increasing activities of the Laboratory, their relationship to each other and to the organization as a whole. The purpose was to establish more clearly than heretofore the lines of authority and responsibility extending from the Executive Committee through the Director and General Manager to the various departments. The recommendations of the Committee were adopted and put into operation with highly satisfactory results.

3. *Increases in Rates and Fees*

A careful analysis of the cost of operating the mess and the residence buildings showed that the rates charged have not been sufficient to cover expenses. The deficits incurred each year had to be paid out of general Laboratory funds. The Executive Committee felt that while research should be subsidized, the cost of running the mess and dormitories should be borne entirely by those who avail themselves of these facilities. The rates were therefore raised by amounts which, it is hoped, will not only wipe out the deficit, but will permit improvements in the service.

4. *Old Main*

The renovation of Old Main, made possible through the generosity of the Rockefeller Foundation, is now complete. An inspection of the building will show how well the Committee in charge of the project, under the energetic Chairmanship of Mr. Claff, has done its work. Careful planning of expenditures permitted the installation of fresh and salt water supplies in every laboratory room, and in addition a new and adequate electric circuit to supply the Mess and other buildings in the area.

5. *Housing*

Many years ago the Laboratory obtained the Gansett and Devil's Lane tracts to provide its members with suitable building lots. In the former area all the lots

have now been sold. In Devil's Lane 23 lots have been purchased and seven houses are either under construction or already completed. Others will be built in the near future. The Town of Falmouth has taken over the roads which had been laid out, and will be responsible for their maintenance. Water mains have also been laid, and electricity will be available in the summer of 1950.

6. *Gifts*

The Laboratory acknowledges with gratitude the gift of a boat, presented by Mrs. John G. Ralston of Dixon, Illinois. This craft, now appropriately named "Arbacia," has been equipped with light dredging gear and will be used in a biological survey of this area. It is fast, seaworthy, and can accommodate a crew which may find it necessary to spend several days away from Woods Hole on extended collecting trips.

Dr. William D. Curtis gave $400 to be used for elevating the roof of the pilot house on the "Dolphin."

The Associates of the Marine Biological Laboratory contributed $1,370 for the purchase of research apparatus.

The Rockefeller Foundation gave $4,905.44 to purchase equipment to be used by the Institute of Muscle Research under the direction of Dr. Szent-Gyorgyi.

7. *The F. R. Lillie Memorial Research Fund*

The appeal for contributions to the F. R. Lillie Memorial Research Fund has resulted in a generous response from members of the Corporation and from friends. In addition, Dr. Clowes, Chairman of the Committee in charge of the drive, has been successful in obtaining substantial gifts. Donations received from members will be used for the erection of a bronze tablet to be placed in the lobby of the Brick Building. Together with the present Agassiz and Whitman tablets it will be a part of a group which will emphasize the service of these three great scientists in the initiation, direction, and development of the Marine Biological Laboratory. The larger part of the fund will be used for general research purposes, including the biological survey.

8. *The Biological Survey*

The need for a new biological survey of this region has been repeatedly emphasized in recent years. Plans for a long range project are under consideration, but in the meantime, the finding of adequate sources of sea urchins is of immediate practical importance. This was shown by the experience of last summer when, for a time, no urchins could be obtained. Under the direction of Mr. McInnis, Vineyard Sound will be thoroughly explored. Later, the area to the westward will be worked over. The results of these operations are shown on a chart in the Committee Room. Our Naturalist, Dr. Rankin, has been placed in charge of the more general survey.

9. *Instruction*

Dr. Frank A. Brown, Jr., resigned after completing five years of most successful service as head of the course in Invertebrate Zoology. Under his direction the

present staff, with some assistance from former staff members and others, have published a laboratory guide, "Selected Invertebrate Types." The book is extensively illustrated, and in condensed form brings together the content of many monographs, and other material not in monograph form. In the introduction Dr. Brown expresses the hope, in which we all share, that "the publication of the volume will result in a rejuvenation in the teaching of this important subject which provides so much promise in the solution of virtually every basic problem in biology and medicine."

Dr. Lewis H. Kleinholz, Professor of Biology at Reed College, was appointed to take charge of the course in place of Dr. Brown.

10. *Deaths*

The Laboratory has lost one of its most distinguished members, Dr. Leonor Michaelis, whose scientific contributions based on work done here, added much to the fame of this institution. His lucid discussions at the Friday Evening Lectures and before the Physiology Class will be long remembered.

11. *Election of Trustees*

At the Annual Meeting of the Corporation held August 9, 1949, the following were elected Trustees of the Marine Biological Laboratory:

8 Trustees—Class of 1953

W. R. Amberson	R. Chambers
P. B. Armstrong	C. L. Prosser
E. G. Ball	A. C. Redfield
F. A. Brown, Jr.	A. Tyler

12. *There are appended as parts of this report:*

1. Memorials
2. Appreciation of the services of Mr. Frank M. MacNaught
3. The Staff
4. Investigators and Students
5. The Lalor Fellows
6. The Atomic Energy Fellows
7. Tabular View of Attendance, 1945–1949
8. Subscribing and Cooperating Institutions
9. Evening Lectures
10. Shorter Scientific Papers presented at the Seminars
11. Members of the Corporation

Respectfully submitted,

CHARLES PACKARD,
Director

1. Memorials

Sumner Cushing Brooks, 1888–1948

By

H. F. Blum

In the death of Sumner Cushing Brooks in Bermuda on April 23rd, 1948, the Marine Biological Laboratory lost a staunch friend as well as a distinguished scientist. His unflagging interest in the laboratory is testified to by the regularity of his summer attendance here, even after he took up his residence in 1928 on the other side of the continent. His first summer as independent investigator was in 1919, his last in 1946, although he paid a brief visit here in 1947. His wife, Matilda Moldenhauer Brooks, usually accompanied him, sharing a laboratory in which they worked on problems often closely allied. Brooks became a member of the Corporation in 1923, and was elected a Trustee in 1941.

Although born in Japan, Brooks could claim only very short residence there. The accident of his birthplace resulted from his father's position as Professor at the Imperial College of Agriculture, which he relinquished a few months after Sumner's birth to accept a professorship at Massachusetts Agricultural College.

Sumner Brooks took his bachelor's degree at the latter institution in 1910, and remained there afterwards as an assistant in Botany. He obtained his Ph.D. in the same subject from Harvard in 1916. Subsequently he held various positions, including six years in the Hygienic Laboratory of the U. S. Public Health Service, and professorships at Bryn Mawr, Rutgers, and California.

Sumner Brooks loved to travel, and this urge took him to many parts of the earth. Often his journeys had as their chief aim, experimental studies which could best be carried on where the particular biological material was available; but wherever he went he was an avid student of nature and a careful observer of whatever came within his view, particularly bird life. I remember, for example, his account of timing the wing beats of albatross and correlating this with latitude as he came home from the South Seas to Berkeley. Such observations were seldom if ever published—this was pure avocation, stemming from his keen enjoyment of nature. His interest in birds was with him when he moved from the east coast to the west, where he was soon associated with an active group of ornithologists, and at the time of his death he was an officer in the Cooper Ornithological Society.

Brooks' scientific achievements, particularly in the field of permeability, need no review for this audience. His researches were always attempts to probe into fundamental problems of cellular physiology, rather than applications of existing biological knowledge. He was a general physiologist in the broadest sense, and when the formation of a Society of General Physiologists was undertaken here, in 1946, he, of course, took an active part.

Although suffering severe physical handicaps during the last years of his life, he continued until the end his interest in biology inside and outside the laboratory. His death came quickly, and as he might have wished—during a period of investigation, characteristically far from home.

Selig Hecht, 1892–1947

By

Otto Glaser

Selig Hecht arrived at Woods Hole in 1918. It was five years after his graduation from the City College of New York, where he had studied chiefly Physics, Chemistry, and Mathematics, but where also, and with rare perception, George Gilmore Scott had

interested his pupil in measurements on dogfish. Two seasons at Beaufort, 1912 and 1913, produced additional measurements on 12 species of teleost. Perhaps most decisive at this stage of Hecht's career was the display of mathematically definable order by quantitative data on various dimensions of an organism and the obvious relevance of the measurements to one another. In 1916 a third paper, "Form and Growth of Fishes," comments on the use of volumes and especially weights as criteria of growth and demonstrates how fluent he had become in the idiom of biology. "The usefulness of an organ," he wrote, "and the adaptedness of an organism to its environment, are hardly functions of their weight. The form, however, is of prime importance. Sixteen years later this work was assigned its place in what is now called Allometry.

Before entering Harvard in 1915, Hecht served as a Pharmacologist in the department of Agriculture and in one year saved enough money to begin graduate study. A doctoral thesis prepared under George Howard Parker took Hecht to Bermuda. "The Physiology of *Ascidia atra*" was published in 1918, part of the thesis having won the Bowdoin Prize in 1917 "for essays of high literary merit." These observations and experiments included, incidentally, the qualitative basis for all his later work.

Fresh from a rigorous quantitative analysis of photic responses in *Ciona intestinalis*, studied at La Jolla, Hecht knew precisely why he came to Woods Hole. He had postulated a simple mechanism. A photo-sensitive material is present in the Ciona siphons. Under the influence of light this substance breaks down into two others whose reunion restores the original photosensitive material. Here was the first version of a model destined for some changes and numerous applications.

Between 1918 and 1924, the year of his election to our Corporation, Hecht tested this hypothesis with the utmost care on the clam, *Mya arenaria*. From conversations and the nature of the experiments on Mya, it was apparent that he expected to study vision, but also that he intended to rest his case among organisms where photoreception is uncomplicated by psychological factors or even optics. About sixteen publications dealt with the duration of the latent period; with the stationary state; with intensity and photoreception; with the kinetics of dark adaptation; with time, intensity, and wave length, in photic excitation; and with the applicability of the Bunsen-Roscoe law to minimal and other energy levels.

During this period Hecht labored in the catacombs then under Old Main and frequently by accident but sometimes to relieve his maddening migraine, whacked his head against the rafters. In those days he spoke poignantly about three books that had captivated his imagination. One was George Moore's "The Brook Kerith"—reread several times and always with tears over the same passages. But there were also the concise "Quantitative laws in biological chemistry" by Arrhenius, and Mellor's "Higher mathematics for students of chemistry and physics." From Arrhenius he absorbed the idea of using temperature as a tool for the interpretation of biological processes in terms of chemical reaction kinetics. From Mellor and more specialized sources he acquired the technique and insights that enabled him to extract meaning, implications and the implications of these, from his meticulous data and his meticulously drawn curves. A curve was more than a graphic representation. It was something to "stew" over until its naive statements and their direct and indirect consequences crystallized into a system internally consistent and congruous with other data, either known or obtainable by further experimentation. In the atmosphere of that decade, facile taxonomists classified Hecht's rationalism and sophisticated methodology mostly as witchcraft.

By 1921 a fourth year had rolled by as Assistant Professor of Biochemistry in the Creighton University Medical School in Omaha, Nebraska. Seconded by two powerful friends, Jacques Loeb and Thomas Hunt Morgan, Hecht then embarked on a series of National Research and General Education Board Fellowships. For another five years these enabled him to work now with Henderson at Harvard, now with Baly or Barcroft

in England; to travel on the continent, and to spend a year at Naples. By 1926 he was appointed Associate Professor of Biophysics at Columbia and two years later rose to full professorship. After 1924 he did not return to Woods Hole until 1927 and after 1929 came only for short visits. Between these last dates he worked in room 231 of the Brick Building and devoted his time largely to the effects of intermittent light; produced his theoretical derivation of Talbot's Law, and a validation of the theory on Mya.

Hecht's entire scientific development is an illustration of intellectual orthogenesis. Even before his foundations had solidified in detail, he used Ciona and Mya as guides to dark adaptation and intensity discrimination in man. His prolonged and incomparable exploration of the human retina began in 1920. These investigations quickly uncovered objective criteria for distinguishing rod from cone vision. His dramatic demonstration that curves depicting the relation between intensity and visual acuity are population curves reflecting differences in the sensitivity of individual rods and cones, and also the number of such receptors active at given intensities, was presented in a memorable Friday Evening lecture in 1924. Later the underlying idea was tested more directly on the eyes of fruit flies and bees where the same relations hold and where the number of functional ommatidia can be easily counted.

Beginning also in 1920, and interspersed with the researches of the next 22 years, were experiments on the photochemistry of Visual Purple. Here temperature effects on the rate of bleaching indicated a first order reaction directly proportional to intensity and with no thermal increment. With the assistance of Chase, Schlaer and Haig this phase culminated in the verification, long overdue, of Kühne's reported regeneration of Visual Purple in solution. The Ciona model had found its replica in the vertebrate eye.

From the outset Hecht brooded over the perennially difficult problems of color vision, but felt unprepared. In another 12 papers he reexamined every relevant aspect of his foundations. At Naples in 1925, he reinvestigated Ciona and added the lamellibranch Pholas to his small repertoire of animals. He was now postulating for Ciona, Mya and Pholas photoreceptive processes identical in architecture but different in their chemical components.

The frontal attack on Color Vision began in 1928. Characteristically, Hecht established almost at once the essential formal adequacy of Young's three-fiber theory. This was indeed capable of yielding a quantitative formulation of Color Vision. Nevertheless, retaining only the structural intuition in Young's view and integrating this with the Helmholtz three-substance theory, he decided in favor of a three-cone hypothesis. This became basic for his analysis of the several types of color-blindness.

After night-blindness had been associated by others with deficiency or absence of Vitamin A, and Wald had identified both Vitamin A and the carotenoid retinene in normal retinas, Hecht instigated five special studies on dark adaptation and from the general similarities between rod and cone vision, indicated that Vitamin A is probably present also in cones. In cooperation with Mandelbaum, a study of Vitamin A and Rod-Cone adaptation in cases of cirrhosis of the liver was followed by the development of a diagnostic method for determining Vitamin A deficiencies.

Early in the Mya era, Hecht had dealt with minimal responses in relation to minimal energy levels. Twenty years later, after a study of night vision in the owl, he undertook from an entirely different standpoint his Quantum Analysis of Vision. Given the discontinuities of light, the discontinuities of the retinal receptors, and certain ingenious corrections for reflection and absorption by the non-photosensitive ocular media, he succeeded in finding what proportion of incident light actually reaches the retina. In order to see, a minimum of from 5 to 14 rods must each absorb one quantum of light. At this level he maintained conventional explanations for variability of behavior breakdown for here the stimulus is less accurately controlled than the organism.

Hecht's output includes about twenty-two communications of a general nature. Some of these, notably his first papers on temperature, on Young's theory, and his deriva-

tion of Talbot's Law, suggest the moods of the theoretical physicist. About fifteen others are lectures, contributions to symposia, critical summaries and reviews. In this family of papers, Hecht kept up with himself, tightening his hold by keeping all parts of his field freshly before his mind.

The audiences he addressed differed widely. Some were essentially lay; others were Ophthalmologists, Clinicians, Physicists, Chemists, or Biologists. Mostly in English, the list also includes German, French and even Russian items. The longest, with 47 illustrations, is the great Summary in the *Asher-Spiro Ergebnisse* for 1931, which appeared later in book form in French. Repetitions were, of course, unavoidable and on occasion he followed verbatim earlier statements that he could not improve. Yet, in general, each attempt to reach a different group involved a fresh effort at simplification and intelligibility. Concerned always with the historical development of his subject he frequently referred to contributions over 100, and even 226 years old. From the Classics, especially Koenig and Brodhun, 1889, he recovered usable measurements not influenced by his own ideas and hence sources of the greatest satisfaction. Unless editorial policies over-ruled, his bibliographies were unscathed by the drought that dries the streamlined reference lists. He always gave the full title of every citation.

With our entry into the war the practical value of Hecht's experience became at once obvious. Under the Division of Medical Sciences of the National Research Council, he joined the sub-committee on Visual Problems and under the O.S.R.D. became an official investigator both for the Army and the Navy. Promptly Hecht and his colleagues were swamped by Government contracts entailing special laboratory and field investigations. More grinding experiences were provoked by the paper work, and the innumerable discussions with Officers of the Armed Forces. When not flying to Camp LeJeune in North Carolina or to Milwaukee, Hecht oscillated between Bridgewater, Connecticut, and New York or between New York and Washington. Only a few of the results have been printed, *e.g.*, on the relation between Anoxia and Brightness Discrimination; on size, shape, and contrast in the Detection of Targets by Daylight and on Dark Adaptation following Light Adaptation to Red and White Lights. To this period belongs also a popular article in Harper's magazine on "Seeing in a black out."

For Biology in general, it is unfortunate that the four large volumes of the Hechtian Corpus should be almost exclusively the possession of specialists. Here we have one of the best examples to show how a limited acreage cultivated intensively may produce flowers of research able to pollinate adjacent fields and also to set the fruits required for self-propagation or available for immediate consumption in cases of need. More widely appreciated, the logical sequence of these investigations and the special logic of each installment, could only raise the level of scientific writing. His style permitted revealing comments and asides. "The essential function of vision is to explore the environment by the use of light"; "My purpose is not to add to the literature but rather to subtract from it"; "Experiments are exciting in proportion to the ideas which determine their existence"; "The ideas themselves must be considered merely as quantitative experiments in thinking about Color Vision," are all trenchant sentences. Hecht unmasked more completely during a joint meeting of the Optical Society and the Society of Rheology. In reporting his Quantum Studies he begins: "I would like to lay before you more than the data and conclusions. If possible I want to convey something of the pleasure with which this research has been associated, something of the intellectual excitement which surrounded its progress. Researches vary in this respect but of all those in which I have engaged none has carried such a consistent accompaniment of delight and I hope some of this may be carried over in the presentation."

All his writings unfold the teacher who could take his learning lightly yet with no trace of flippancy, or clowning. We recall a charming but profound essay on Human Behavior and the Uncertainty Principle printed in Harper's Magazine and editorialized

in the New York Times. Certainly "Explaining the atom" is another case in point. The most satisfactory of a group of popular books on atomic energy, it had a surprising sale, was adopted as a text by the Armed Forces and explains his presence on the Committee of Atomic Scientists.

In conventional teaching he was extraordinarily effective. The impressionism that gave such flare to hundreds of his watercolors bespoke a deep seated temperamental diathesis. In science, too, he could hack a trail through jungles of detail and irrelevancies, irksome, but not invariably as innocent as he thought. Always he reached a clearing. At the New School for Social Research he impressed Alvin Johnson as the best teacher he had ever known.

Hecht was one of those about whom the hours seem to hover in their flight. Preoccupied as he was, he always had time to paint and sing; time for literature, concerts, and conversations; time also to run a summer school for children who repeated the classical experiments of Archimedes, Galileo and Newton in a Vermont barn with the simplest of improvised equipment. Of himself he gave lavishly. Criticisms, suggestions, help with the experiments of others and with mathematical interpretations were available in inexhaustible supply. He conducted a lively correspondence. Still his energies were unspent. He revived and edited the Columbia Biological Series. His services on the Editorial Boards of the *Biological Bulletin*; of the *Journal of the Optical Society of America*; of the *Monographs of Experimental Biology*; and of the *Documenta Ophthalmologica* were additional outlets to regulate the creative pressure.

It is difficult to realize that one who was to cherish the Ives Medal of the Optical Society, the Townsend Harris Medal of the City College of New York and election to the National Academy of Sciences could, in 1917, find no proper niche for his unique constellation of talents. Are there comparable instances today? During the decade between the doctorate and his Professorship, he depended heavily on a few friends and on the constant sympathy, encouragement and sacrifices of his wife, Celia Hecht. Why were these the only human beings to recognize the early symptoms of his great potentialities? That one so vital could be struck down with all his powers still at flood tide remains almost incredible to those who, in fancy, can still hear these corridors reverberate with his impassioned argument and his Homeric laughter.

2. In Appreciation

The following minute, prepared by Dr. H. H. Plough, was adopted by the Trustees at the meeting held in New York City on December 29, 1949:

On January 1, 1950, Frank M. MacNaught will retire as business manager of the Marine Biological Laboratory. When he thus attains *emeritus* status, Mr. MacNaught will have served the laboratory for thirty-seven years in what is in many respects the most important position on the laboratory staff. During that period he has come to know more members and guests of the laboratory than any other officer, excepting only his chief associate, Miss Polly Crowell. There are none of this long list but bear for Mr. MacNaught not only respect but warm personal regard.

Mr. MacNaught was appointed as the sole member of the business staff in 1913. The writer of this minute was a student in one of the courses in that summer, and he still remembers with pleasure the kindness and friendliness shown by Mr. MacNaught to a young college student who was unfamiliar with the arrangements at the laboratory. His unique gift of friendliness, his ability to treat each member of the laboratory as though his needs were paramount, his willingness to spend time with each one personally until, within the range of the possible, his needs were satisfied—these human qualities have endeared Mr. MacNaught to hundreds of Woods Hole visitors. They will hope to see him each summer even if he turns over his official duties to younger hands.

The work of Mr. MacNaught rapidly grew in the years following his appointment. During the term of Frank R. Lillie as director, it was realized that two sorts of service were required in the office and he took them both over. One had to do, as originally contemplated, with keeping the accounts of the laboratory under the advice of the Director for consideration of the Executive Committee. In another respect, however, service as registrar was required—including not only arrangements for laboratory space and course tables, but the even more difficult responsibility of arranging and assigning housing, and of taking responsibility for the mess and its many temporary employees. All of these many tasks were gradually consolidated in Mr. MacNaught's capable hands and he was, by appointment of the Executive Committee, made Business Manager in 1916.

Since then he has served under two directors following Dr. Lillie—Drs. Jacobs and Packard, and has continued to merit the confidence and cooperation of each.

Both Trustees and Staff congratulate Mr. MacNaught on a unique and valuable contribution to the Marine Biological Laboratory, and hope they may continue to call him fellow member and friend.

For the Trustees,
WILLIAM R. AMBERSON,
Secretary of the Board

3. THE STAFF, 1949

CHARLES PACKARD, Director, Marine Biological Laboratory, Woods Hole, Massachusetts.

SENIOR STAFF OF INVESTIGATION

E. G. CONKLIN, Professor of Zoology, *Emeritus*, Princeton University.
W. E. GARREY, Professor of Physiology, *Emeritus*, Vanderbilt University Medical School.
RALPH S. LILLIE, Professor of General Physiology, *Emeritus*, The University of Chicago.
A. P. MATHEWS, Professor of Biochemistry, *Emeritus*, University of Cincinnati.
G. H. PARKER, Professor of Zoology, *Emeritus*, Harvard University.

ZOOLOGY

I. CONSULTANTS

LIBBIE H. HYMAN, American Museum of Natural History.
A. C. REDFIELD, Woods Hole Oceanographic Institution.

II. INSTRUCTORS

F. A. BROWN, JR., Professor of Zoology, Northwestern University, in charge of course.
W. D. BURBANCK, Professor of Biology, Drury College.
C. G. GOODCHILD, Professor of Biology, S.W. Missouri State College.
L. H. KLEINHOLZ, Associate Professor, Reed College.
JOHN H. LOCHHEAD, Assistant Professor of Zoology, University of Vermont.
MADELENE E. PIERCE, Associate Professor of Zoology, Vassar College.
W. M. REID, Professor of Biology, Monmouth College.
T. H. WATERMAN, Assistant Professor in Biology, Yale University.

III. LABORATORY ASSISTANTS

R. S. HOWARD, University of Miami.
MARIE WILSON, Northwestern University.

EMBRYOLOGY

I. Instructors

Donald P. Costello, Professor of Zoology, University of North Carolina, in charge of course.
William W. Ballard, Professor of Zoology, Dartmouth College.
Arthur L. Colwin, Assistant Professor of Zoology, Queens College.
Charles B. Metz, Assistant Professor of Zoology, Yale University.
James A. Miller, Associate Professor of Anatomy, Emory University.
Albert Tyler, Associate Professor of Embryology, California Institute of Technology.

II. Research Assistant

Margaret E. M. Davidson, McGill University.

III. Laboratory Assistants

Helen A. Padykula, Mount Holyoke College.
James M. Moulton, Harvard University

PHYSIOLOGY

I. Consultants

Eric G. Ball, Professor of Biochemistry, Harvard University Medical School.
Merkel H. Jacobs, Professor of Physiology, University of Pennsylvania.
Otto Loewi, Professor of Pharmacology, New York University, School of Medicine.
Arthur K. Parpart, Professor of Biology, Princeton University.

II. Instructors

E. S. Guzman Barron, Associate Professor of Biochemistry, The University of Chicago, in charge of course.
M. J. Kopac, Associate Professor of Biology, New York University.
Hans Neurath, Professor of Biochemistry, Duke University Medical School.
Robert F. Pitts, Professor of Physiology, Syracuse University, College of Medicine.
H. Burr Steinbach, Professor of Zoology, University of Minnesota.
George Wald, Professor of Biology, Harvard University.

BOTANY

I. Consultants

Bostwick H. Ketchum, Woods Hole Oceanographic Institution.
Wm. Randolph Taylor, Professor of Botany, University of Michigan.

II. Instructors

Maxwell S. Doty, Assistant Professor of Botany, Northwestern University. In Charge of Course.
Edwin T. Moul, Instructor in Botany, Rutgers University.
R. D. Wood, Assistant Professor of Botany, Rhode Island State College.

III. Research Assistant

Justine Garnic, Carnegie Institute of Technology.

IV. Laboratory Assistant

Albert J. Bernatowicz, University of Michigan.

V. Lecturers

J. B. Lackey, Philadelphia, Pa.
Ruth Patrick, Curator of Limnology, Academy of Natural Sciences of Philadelphia.

VI. Field Consultant and Collector

Hannah T. Croasdale, Research Associate, Dartmouth College.

EXPERIMENTAL RADIOLOGY

G. Failla, College of Physicians and Surgeons, Columbia University.
L. Robinson Hyde, Phillips Exeter Academy, Exeter, N. H.

LIBRARY

Deborah Lawrence, Librarian
Margaret P. McInnis — Mary A. Rohan
Jean Goodfellow

APPARATUS DEPARTMENT

Robert B. Mills, Manager
J. D. Graham — Dorothy LeFevre
E. P. Little, Consultant
Seaver R. Harlow, Machinist

CHEMICAL DEPARTMENT

Robert B. Mills, Manager

SUPPLY DEPARTMENT

James McInnis, Manager
John S. Rankin, Naturalist

Ruth Crowell		Marcia McLaughlin
M. B. Gray	W. E. Kahler	R. E. Tonks
A. M. Hilton	Carl O. Schweidenback	F. N. Whitman
	G. Lehy	

GENERAL OFFICE

F. M. MacNaught, Business Manager
Homer P. Smith, Assistant Business Manager
Polly L. Crowell — Mrs. Lila S. Myers
Norma Ferreira

GENERAL MAINTENANCE

R. W. Kahler, Manager

Robert Adams	A. Neal
R. Gunning	G. T. Nickelson, Jr.
J. H. Head	A. J. Pierce
G. A. Kahler	T. E. Tawell

4. Investigators and Students

Independent Investigators, 1949

Abrams, Richard, Assistant Professor, University of Chicago.
Allen, Ezra, Visiting Professor of Biology, Stetson University.
Allen, M. Jean, Instructor in Zoology, University of New Hampshire.
Alscher, Ruth P., Instructor, Manhattanville College.
Amberson, William R., Professor of Physiology, University of Maryland Medical School.
Anderson, Rubert S., Professor of Physiology, University of South Dakota.
Atwood, Kimball C., Research Associate in Microbiology, Columbia University.
Baily, Norman A., Research Scientist, Columbia University.
Ball, Eric G., Professor of Biological Chemistry, Harvard Medical School.
Ballard, W. W., Professor of Biology, Dartmouth College.
Barron, E. S. Guzman, Associate Professor of Biochemistry, The University of Chicago.
Berger, Charles A., Director, Biological Laboratory, Fordham University.
Bernstein, Maurice H., Graduate Assistant, Washington University.
Bliss, Alfred F., Associate Professor of Physiology, Tufts College Medical School.
Blum, Harold F., Physiologist, Princeton University.
Bridgman, Anna J., Professor of Biology, Limestone College.
Brooks, Matilda M., Research Associate in Biology, University of California.
Brown, Frank A., Jr., Professor of Zoology, Northwestern University.
Browning, Iben, National Research Fellow, University of Pennsylvania.
Brust, Manfred, Research Assistant in Physiology, University of Chicago.
Burbanck, W. D., Chairman, Department of Biology, Drury College.
Butler, Elmer G., Professor of Biology, Princeton University.
Chase, Aurin M., Associate Professor of Biology, Princeton University.
Cheney, Ralph H., Professor of General Physiology, Brooklyn College.
Claff, C. Lloyd, Research Associate in Surgery, Harvard Medical School.
Clark, Arnold M., Professor of Biology, University of Delaware.
Clark, Eliot R., Professor of Anatomy, University of Pennsylvania School of Medicine.
Clark, Leonard B., Chairman, Department of Biology, Union College.
Clement, A. C., Professor of Biology, College of Charleston.
Clowes, G. H. A., Research Director *Emeritus*, Lilly Research Laboratories.
Cohen, Arthur I., Teaching Assistant in Zoology, University of Minnesota.
Cohen, Isadore, Associate Professor, American International College.
Cole, Kenneth S., Scientific Director, Naval Medical Research Institute.
Colwin, Arthur L., Assistant Professor of Biology, Queens College.
Conklin, E. G., Professor of Biology *Emeritus*, Princeton University.
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Costello, Donald P., Professor of Zoology, University of North Carolina.
Cotzias, George C., Assistant Physician, Rockefeller Institute.
Coyle, Elizabeth E., Associate Professor of Biology, College of Wooster.
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Dahl, A. Orville, Chairman, Department of Botany, University of Minnesota.
DeLamater, Edward D., Associate Research Professor of Dermatology, University of Pennsylvania.
Dent, J. N., Associate Professor of Biology, University of Virginia.
Diller, Irene C., Research Cytologist, Institute for Cancer Research.
Diller, William F., Assistant Professor of Zoology, University of Pennsylvania.
Dixon, June A., Graduate Assistant in Zoology, Washington University.
Doty, Maxwell S., Assistant Professor of Biology, Northwestern University.
Driscoll, Dorothy H., Instructor, Smith College.
Duryee, William R., Cytologist, Carnegie Institution.
Failla, G., Professor of Radiology, Columbia University.
Ferguson, Frederick P., Assistant Professor of Physiology, University of Maryland Medical School.

FOGELMAN, M. J., Fellow in Neurosurgery, Southwestern Medical College.
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GAFFRON, HANS, Associate Professor of Biochemistry, University of Chicago.
GARREY, W. E., Professor of Physiology *Emeritus,* Vanderbilt University School of Medicine.
GILMAN, LAUREN C., Associate Professor of Zoology, University of Miami.
GLASER, OTTO C., Professor of Biology, Amherst College.
GOODCHILD, CHAUNCEY G., Professor of Biology, South West Missouri State College.
GOULD, HARLEY N., Professor of Biology, Newcomb College, Tulane University.
GRAND, C. G., Research Associate, New York University.
GREEN, JAMES W., Assistant Professor of Physiology, Rutgers University.
GREENBERG, RUVEN, Instructor of Physiology, Ohio State University.
GROSCH, DANIEL S., Assistant Professor of Zoology, North Carolina State College.
GRUNDFEST, HARRY, Associate Professor of Neurology, Columbia University.
HARRIS, DANIEL L., Assistant Professor of Physiology, University of Chicago.
HARVEY, ETHEL BROWNE, Independent Investigator of Biology, Princeton University.
HARVEY, E. NEWTON, Professor of Physiology, Princeton University.
HAXO, FRANCIS, Instructor in Biology, Johns Hopkins University.
HAYWOOD, CHARLOTTE, Professor of Physiology, Mount Holyoke College.
HEIDENTHAL, GERTRUDE, Associate Professor of Biology, Russell Sage College.
HEILBRUNN, L. V., Professor of Zoology, University of Pennsylvania.
HENLEY, CATHERINE, Research Assistant, University of North Carolina.
HICKSON, ANNA KELTCH, Research Chemist, Lilly Research Laboratories.
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HUNTER, F. R., Associate Professor of Physiology, University of Oklahoma.
IRVING, LAURENCE, Consultant, U. S. Public Health Service.
JACOBS, M. H., Professor of General Physiology, University of Pennsylvania.
JENKINS, GEORGE B., Professor of Anatomy *Emeritus,* George Washington University.
JEPPS, MARGARET W., Glasgow University.
KAAN, HELEN W., Research Associate, National Research Council.
KEMPTON, RUDOLF T., Professor of Zoology, Vassar College.
KESTON, ALBERT S., Assistant Professor of Chemistry, New York University.
KIND, C. ALBERT, Assistant Professor of Chemistry, University of Connecticut.
KISCH, BRUNO, Professor of Chemistry, Yeshiva University.
KITCHIN, I. C., Associate Professor of Biology, University of Georgia.
KLEINHOLZ, LEWIS H., Associate Professor of Biology, Reed College.
KLOTZ, I. M., Associate Professor of Chemistry, Northwestern University.
KRAHL, MAURICE E., Associate Professor of Biochemistry, Washington University.
KUFFLER, STEPHEN W., Assistant Professor of Physiological Optics, Johns Hopkins Medical School.
LAJTHA, ABEL, Institute for Muscle Research.
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LANSING, ALBERT I., Associate Professor, Washington University School of Medicine.
LEFEVRE, PAUL G., Assistant Professor of Physiology, University of Vermont College of Medicine.
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LILLIE, RALPH S., Professor of Physiology *Emeritus,* University of Chicago.
LOCHHEAD, JOHN H., Assistant Professor of Zoology, University of Vermont.
LOVELACE, ROBERTA, Adjunct Professor of Biology, University of South Carolina.
LUCKE, BALDUIN, Professor of Pathology, University of Pennsylvania.
LUYET, B., Professor of Biophysics, Saint Louis University.
MARMONT, GEORGE H., Assistant Professor of Physiology, University of Chicago.
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MCCOLL, J. D., University of Western Ontario Medical School.
MENKIN, VALY, Associate Professor of Experimental Pathology, Temple University Medical School.
METZ, CHARLES B., Assistant Professor of Zoology, Yale University.
MIHALYI, ELEMER, Institute for Muscle Research.
MILLER, JAMES A., Associate Professor of Anatomy, Emory University.
MILNE, LORUS J., Associate Professor of Zoology, University of New Hampshire.
MOORE, GEORGE M., Associate Professor of Zoology, University of New Hampshire.
MOUL, EDWIN T., Assistant Professor of Botany, Rutgers University.
MUSACCHIA, X. J., Instructor, Saint Louis University.
NACHMANSOHN, DAVID, Assistant Professor of Neurology, Columbia University.
NELSON, LEONARD, Instructor in Zoology, University of Nebraska.
NEURATH, HANS, Professor of Physical Biochemistry, Duke University School of Medicine.
NOLAND, JERRE L., Research Assistant in Biochemistry, University of Wisconsin.
O'BRIEN, JOHN A., Assistant Professor of Biology, Catholic University of America.
O'BRIEN, JOHN P., Assistant Professor of Zoology, Marquette University.
OSTERHOUT, W. J. V., Member *Emeritus*, Rockefeller Institute.
PALAY, SANFORD L., Instructor in Anatomy, Yale University School of Medicine.
PARMENTER, CHARLES L., Professor of Zoology, University of Pennsylvania.
PARPART, ARTHUR K., Chairman, Department of Biology, Princeton University.
PARSHLEY, HOWARD M., Professor of Zoology, Smith College.
PICK, JOSEPH, Associate Professor in Anatomy, New York University College of Medicine.
PIERCE, MADELENE E., Associate Professor of Zoology, Vassar College.
PLOUGH, HAROLD H., Professor of Biology, Amherst College.
PLUMMER, JEWEL L., Teaching Fellow, New York University.
PROSSER, C. LADD, Professor of Zoology, University of Illinois.
PROVASOLI, LUIGI, Research Associate, Haskins Laboratories.
REID, W. MALCOLM, Head of the Department of Biology, Monmouth College.
REINER, JOHN M., Research Associate, Tufts College Medical School.
REINHARD, E. G., Head of the Department of Biology, Catholic University of America.
RIESER, PETER, University of Pennsylvania.
ROSE, S. MERYL, Associate Professor of Zoology, Smith College.
ROSENTHAL, THEODORE B., Research Associate, Washington University Medical School.
ROSSI, H. H., Physicist, Department of Radiology, Columbia University.
ROTH, JAY S., Assistant Professor of Biochemistry, Rutgers University.
ROTHENBERG, MORTIMER A., Columbia University.
ROY, S. S., Lecturer, Calcutta University.
RUDENBERG, FRANK H., Harvard University.
RUGH, ROBERTS, Associate Professor of Radiology, Columbia University.
SARKAR, NIRMAL, Lecturer in Chemistry, Calcutta University.
SCHAEFFER, A. A., Professor of Biology, Temple University.
SCHALLEK, WILLIAM B., Assistant Professor of Biology, University of Oregon.
SCHMITT, FRANCIS O., Head of the Department of Biology, Massachusetts Institute of Technology.
SCHMITT, OTTO H., Professor of Zoology, University of Minnesota.
SCHOLANDER, P. F., Swarthmore College.
SCOTT, ALLAN C., Associate Professor of Biology, Union College.
SCOTT, SISTER FLORENCE MARIE, Professor of Biology, Seton Hill College.
SCOTT, GEORGE T., Associate Professor of Zoology, Oberlin College.
SEAMAN, GERALD R., Research Fellow, Fordham University.
SHANES, A. M., Associate Professor of Physiology, Georgetown University School of Medicine.
SICHEL, F. J. M., Professor of Physiology, University of Vermont College of Medicine.
SLIFER, ELEANOR H., Assistant Professor of Zoology, State University of Iowa.
SPEIDEL, CARL C., Professor of Anatomy, University of Virginia.
STEINBACH, H. B., Professor of Zoology, University of Minnesota.
STOKEY, ALMA G., Professor of Botany *Emeritus*, Mount Holyoke College.

STOUDT, H. N., Instructor in Biology, Temple University.
STRAUS, WILLIAM L., Associate Professor of Anatomy, Johns Hopkins University School of Medicine.
STUNKARD, HORACE H., Professor of Biology, New York University.
SUCKLING, EUSTACE E., Instructor, Long Island Medical College.
SZENT-GYORGYI, A. E., Institute for Muscle Research.
TAYLOR, LAURISTON S., Chief Biophysics Branch, Atomic Energy Commission.
TAYLOR, WM. RANDOLPH, Professor of Botany, University of Michigan.
TERRY, ROBERT L., Assistant Professor of Biology, Union College.
TEWINKEL, LOIS E., Associate Professor of Zoology, Smith College.
TING, TE-PANG, Research Associate, Amherst College.
TRACY, HENRY C., Professor of Anatomy, University of Kansas.
TRINKAUS, J. P., Instructor in Zoology, Osborn Zoological Laboratory.
TRUANT, A. P., Assistant Professor of Pharmacology, George Washington University School of Medicine.
TYLER, ALBERT, Associate Professor of Embryology, California Institute of Technology.
VARGA, LASZLE, Institute for Muscle Research.
WALD, GEORGE, Professor of Biology, Harvard University.
WARBURG, OTTO, Visiting Lecturer, Kaiser Wilhelm Institut for Physiology, Berlin.
WARNER, ROBERT C., Assistant Professor of Chemistry, New York University College of Medicine.
WENRICH, D. H., Professor of Zoology, University of Pennsylvania.
WHITING, P. W., Professor of Zoology, University of Pennsylvania.
WICHTERMAN, RALPH, Associate Professor of Biology, Temple University.
WILBER, CHARLES G., Director Biological Laboratories, Saint Louis University.
WILLIER, B. H., Director, Biological Laboratories, Johns Hopkins University.
WILSON, WALTER L., Research Associate, University of Pennsylvania.
WOOD, RICHARD D., Instructor in Botany, Rhode Island State College.
WOODWARD, ALVALYN E., Assistant Professor of Zoology, University of Michigan.
WOODWARD, ARTHUR A., Assistant Professor of Zoology, Brown University.
WRINCH, DOROTHY, Lecturer in Physics, Smith College.
WULFF, VERNER J., Assistant Professor of Zoology, University of Illinois.
ZALOKAR, MARKO, Research Fellow, California Institute of Technology.
ZEUTHEN, ERIK, Lecturer, University of Copenhagen.

Beginning Investigators, 1949

BACON, CHARLES R. T., Student, University of Pennsylvania.
BATTLEY, EDWIN H., Student, Harvard University.
BATTY, THOMAS V., Graduate Student, University of Kansas.
BAUER, MARK H., Graduate Student, Princeton University.
BOYLE, E. MARIE, Science Teacher, The Baldwin School.
CARLSON, FRANCIS D., Instructor, Johns Hopkins University.
COOKSON, BRIAN A., Cancer Research Fellow, University of Pennsylvania.
ESSNER, EDWARD S., Assistant Instructor, University of Pennsylvania.
EVANS, JEANNE F., Student, University of Pennsylvania.
FLOOD, VERONICA M., Junior Biochemist, Argonne National Laboratory.
GAGNON, ANDRE, University of Pennsylvania.
GASVODA, BETTY, Junior Biochemist, Argonne National Laboratory.
GENNARO, JOSEPH F., University of Pittsburgh.
HALABAN, ATIDA, Graduate Student, University of Pennsylvania.
HARDING, CLIFFORD V., University of Pennsylvania.
HARDING, DRUSILLA, University of Pennsylvania.
HAY, ELIZABETH D., Student, Johns Hopkins Medical School.
HIMMELFARB, SYLVIA, University of Maryland Medical School.
HIRSCHFIELD, HENRY I., Research Associate, University of Missouri.
HOFFMAN, JOSEPH F., Graduate Student, Princeton University.

HONEGGER, CAROL M., Instructor, Temple University.
HOPKINS, AMOS L., Graduate Student, University of Pennsylvania.
HOULIHAN, ROBERT K., Graduate Assistant, Boston College.
JACOBS, BERTRAND R., Student, University of Pennsylvania School of Medicine.
KAPLAN, ANN ESTHER, Mount Holyoke College.
KAYHART, MARION, University of Pennsylvania.
KELLY, ELIZABETH M., Graduate Assistant, University of Delaware.
KELLY, JOHN W., Graduate Student, University of Pennsylvania.
KIRSCHNER, LEONARD B., University of Wisconsin.
LOVE, WARNER E., University of Pennsylvania.
MALAN, MARTHA, University of Pennsylvania.
MCCAY, PAUL B., University of Oklahoma.
MCCULLOCH, DAVID, Teaching Fellow, Massachusetts Institute of Technology.
MCKEEHAN, MORRIS S., Zoology Assistant, University of Chicago.
MILLER, TILFORD D., Amherst University.
MITCHELL, RUTH, Columbia University.
MOSKOVIC, SAMUEL, Teaching Fellow, New York University.
MOULTON, JAMES MALCOLM, Teaching Fellow, Harvard University.
O'MALLEY, BENEDICT B., Graduate Student, Fordham University.
PADYKULA, HELEN A., Instructor in Zoology, Wellesley College.
PFISTER, RONALD R., Columbia Medical School.
PROCTOR, NATHANIEL K., Graduate Student, University of Pennsylvania.
RAWLEY, JUNE, Instructor, Kent State University.
SALTZ, MYRON, Student, Amherst College.
SCHREIBMAN, IRVIN, University of Pennsylvania.
SCLUFER, EVELYN, Graduate Student, Bryn Mawr College.
SEKI, SADA L., Graduate Assistant, Mount Holyoke College.
STIEGLITZ, ALICE A., Student, University of Pennsylvania.
STRITTMATTER, CORNELIUS F., Harvard University Medical School.
TIETZE, FRANK, Research Fellow, Northwestern University.
VOGEL, MARTIN L., Amherst College.
WILSON, THOMAS H., Instructor in Physiology, University of Pennsylvania.

Research Assistants, 1949

ABRAMSKY, TESS, Georgetown University.
ANDREWS, J. R., United States Public Health Service.
BENSON, ELEANORE, University of Missouri.
BERNATOWICZ, ALBERT J., University of Michigan.
BLUMENTHAL, GERTRUDE, University of Missouri.
BRUMM, ANNE F., New York University.
BULLOCK, JANE A., University of Oklahoma.
BURKE, DEAN, National Cancer Institute.
CURTIS, PAUL, Oberlin College.
DAVIDSON, MARGARET E., McGill University.
FASS, JEROME S., Rockefeller Institute.
FOLEY, MARY T., Yale University.
FRIEDLER, GLADYS, University of Pennsylvania.
FROMBERG, VIVIAN, Woman's Medical College of Pennsylvania.
GARNIC, JUSTINE, Northwestern University.
GOLDRING, ROBERTA, Vassar College.
GOUREVITCH, HARRY G., University of Chicago.
GRIM, MADELON R., Amherst College.
HOLLAND, BERT, University of Chicago.
HOWARD, ROBERT S., University of Miami.
HSU, DOROTHY L., University of Pennsylvania.
JAFFEE, OSCAR, Graduate School of Arts and Science, New York University.
KUN, ERNEST, University of Chicago.

LEE, LOIS E., Southwest Missouri State College.
LEONARD, LAWRENCE, Haverford College.
LITT, MORTIMER, University of Rochester Medical School.
LOEFFLER, ROBERT J., University of Wisconsin.
LYNN, FRANCIS, Stanford University.
MILLER, FAITH S., Emory University.
MITCHELL, CONSTANCE J., University of Delaware.
MOOS, CARL, Massachusetts Institute of Technology.
ORSKI, BARBARA M., Harvard Medical School.
ROSENBLUTH, RAJA, Columbia University.
SANDEEN, MURIEL I., Northwestern University.
SLATTERY, LEO F., University of Chicago.
STAPLETON, R. H., United States Public Health Service.
STOUT, CAROLYN M., University of Pennsylvania.
TALPEY, WILLIAM B., Washington University.
WALTERS, C. PATRICIA, Eli Lilly and Company.
WEBB, H. MARGUERITE, Northwestern University.
WEBER, PATRICIA, St. Louis University.
WEST, ALICE, Radcliffe College.
WILSON, MARIE, Northwestern University.
WINBLAD, JAMES N., University of Kansas.

Library Readers, 1949

ADLER, FRANCIS H., Professor of Ophthalmology, University of Pennsylvania.
BARTLETT, JAMES H., Professor of Physics, University of Illinois.
BLOCH, ROBERT, Research Associate, Yale University.
BODIAN, DAVID, Associate Professor in Epidemiology, Johns Hopkins University.
CANTONI, G. L., New York University.
CASE, JAMES F., Graduate Student, Johns Hopkins University.
CLENDENNING, KENNETH A., Associate Research Biologist, National Research Council, Canada.
COLLIER, ALBERT, Marine Biologist, Gulf Oil Corporation.
CONKLIN, RUTH E., Professor of Physiology, Vassar College.
CSAKY, TIHAMER Z., Research Associate, Duke Hospital.
DEMPSEY, EDWARD W., Harvard Medical School.
EDSALL, GEOFFREY, Professor of Bacteriology, Boston University School of Medicine.
EICHEL, BERTRAM, Assistant Professor, Rutgers University.
EICHEL, HERBERT J., Teaching Assistant in Physiology, Rutgers University.
FLAGLER, ELIZABETH A., Princeton University.
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KABAT, ELVIN A., Associate Professor of Bacteriology, Neurological Institute.
KARUSH, FRED, Fellow, Sloan-Kettering Institute for Cancer Research.
KELLER, RUDOLPH, Director of Research, Madison Foundation.
KEOSIAN, JOHN, Professor of Biology, Newark College, Rutgers University.
KINDRED, JAMES E., Professor of Anatomy, University of Virginia.
KOZAM, GEORGE, Instructor in Anatomy, New York University.
KRASNOW, FRANCES, Head, Department of Research, The Guggenheim Dental Foundation.
KUFF, EDWARD L., Instructor in Anatomy, Washington University School of Medicine.
LEIKIND, MORRIS C., Head of Biology and Medical Unit, Library of Congress.
LING, GILBERT N., Postdoctorate Fellow, University of Chicago.

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MAYOR, JAMES W., Professor of Biology *Emeritus*, Union College.
MCDONALD, SISTER ELIZABETH SETON, Professor of Biology, College of Mount St. Joseph.
MCLEAN, DOROTHY J., Instructor, Vassar College.
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MORRISON, DAVID, Flight Safety Foundation, Inc.
NADEAU, L. V., Graduate Student, Dominican House of Studies.
PERKINS, JOHN F., JR., Assistant Professor, University of Chicago.
QUASTEL, J. H., Professor of Biochemistry, McGill University.
RENN, CHARLES E., Associate Professor of Sanitary Engineering, Johns Hopkins University.
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ROYS, CHESTER, Graduate Student, University of Iowa.
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SMITH, MARY F., University of Oklahoma.
SULKIN, S. EDWARD, Professor of Bacteriology, Southwestern Medical College.
SUTRO, PETER J., Graduate Student, Harvard University.
TANNENBAUM, STUART, Graduate Student, Columbia University.
THERMAN, P. O., Institute of Pennsylvania Hospital.
THOMSON, BETTY F., Assistant Professor of Botany, Connecticut College.
WAINIO, WALTER W., Assistant Research Specialist, Rutgers University.
WITTENBERG, JONATHAN, Graduate Student, Columbia University.
ZORZOLI, ANITA, Washington University.

Students, 1949

BOTANY

BARKER, FRANCES M., Assistant in Bacteriology, Wellesley College.
BENOIT, RICHARD J., Graduate Student, Yale University.
BUBIENIEC, ERNEST J., Student, Harvard College.
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CONOVER, JOHN T., Student, University of Michigan.
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LEWIN, RALPH A., Graduate Student, Yale University.
LOEFFLER, ROBERT J., University of Wisconsin.
PUTALA, EUGENE C., Student, University of Massachusetts.
RIEGER, CECILIA A., Hofstra College.
SMITH, MARGARET J., Vassar College.
STONIER, TOM T., Student, Drew University.
STRAUS, JACOB, Student, College of the City of New York.

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ABSOLON, KAREL B., June Brown Memorial Fellow in Pathology, Yale University.
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BROOKS, HAROLD K., Graduate Student, Harvard University.
CASE, JAMES F., Graduate Student, Johns Hopkins University.
CHIQUOINE, A. DUNCAN, Cornell University.
COHEN, ADOLPH I., Graduate Student, Columbia University.
DAVIDHEISER, ROGER H., Student, University of New Hampshire.
FINNEGAN, CYRIL V., JR., Research Assistant, University of Notre Dame.
GAGE, MARGARET E., Student, DePauw University.
GUYSELMAN, J. BRUCE, Graduate Assistant, Northwestern University.
HEACOCK, RUTH E., Student, Oberlin College.

KITTS, DAVID B., University of Pennsylvania.
KUTA, VIRGINIA A., Graduate Assistant, DePaul University.
LAWLOR, REV. GEORGE F., Instructor, Boston College.
MCKEEHAN, MORRIS S., Student, University of Chicago.
MIRAND, EDWIN A., Teaching Fellow, Syracuse University.
MONTREUIL, PAUL, McGill University.
NERAD, JOSEPHINE J., Graduate Assistant, DePaul University.
OLIVER, SIDNEY W., Instructor of Biology, University of South Carolina.
ORSI, ERNEST V., Student, Fordham University.
PRESCOTT, DAVID M., Student, Wesleyan University.
QUEN, JACQUES M., Graduate Assistant, Brown University.
ROGERS, KAY T., Research Associate, Harvard University.
SHAW, EDWARD I., Graduate Assistant in Zoology, University of Missouri.
SMOLKER, ROBERT E., Boston University.
TOWNES, PHILIP L., Graduate Student, University of Rochester.
TURNER, RUTH D., Research Assistant, Harvard University.
VINSON, CORDELIA A., Graduate Assistant, University of North Carolina.
VINCENT, WALTER S., JR., Graduate Student, University of Pennsylvania.

PHYSIOLOGY

AVERY, MARY E., Student, Johns Hopkins School of Medicine.
BARTON, JAY, II, Graduate and Research Assistant, University of Missouri.
CAMBEL, PERIHAN, Fellow in Cancer Research, Washington University.
DORAIS, JEANNE M. C., Graduate Assistant, Vassar College.
DURELL, JACK, Harvard University.
GOSSELIN, M. LORRAINE, Technician, Rockefeller Institute for Medical Research.
JACOB, MIRIAM I., Technician, Rockefeller Institute for Medical Research.
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KAHN, ARTHUR J., Research Associate, New York University.
KLEIN, RICHARD, Fellow National Cancer Institute, University of Rochester.
KLINE, RAYMOND F., Graduate Student, University of Maryland Medical School.
KOLIN, ALEXANDER, Assistant Professor of Physics, University of Chicago.
LESSE, HENRY, Student, Jefferson Medical College.
MCINTYRE, PATRICIA A., Student, Johns Hopkins University School of Medicine.
MICHEL, BURLYN E., Student, University of Chicago.
MONTAGUE, LAURA J., Student, Johns Hopkins Medical School.
PASSANO, LEONARD M., Graduate Student, Yale University.
SCHIFFMAN, GERALD, Student, New York University College of Medicine.
SCHWARTZ, MARTIN, Graduate Student, Clark University.
SHENG, GINGER, Student, Columbia University.
SMITH, JAY A., Assistant Professor of Physiology, Chicago Medical School.
SNYDER, ROBERT A., University of Western Ontario.
STADLER, DAVID R., Graduate Student, Princeton University.
SWENSON, PAUL A., Stanford University.
THOMPSON, ALAN M., Student, Iowa State College.
THORNBURG, WAYNE, Student, University of Illinois.
WEIMAR, VIRGINIA L., University of Pennsylvania.

INVERTEBRATE ZOOLOGY

AMBERSON, BARBARA D., Pennsylvania State College.
AMOS, PATSY D., Student, Oberlin College.
ANDERSON, BETSY J., Student, Drury College.
ANDERSON, VICTOR ELVING, Student, University of Minnesota.
AUERBACH, EARL, Graduate Student, Northwestern University.
BARBER, SAUL B., Graduate Student, Yale University.
BELLISARIO, JOSEPH, Laboratory Instructor, Washington and Jefferson College.

Birch, Robert Lee, Instructor in Zoology, West Virginia University.
Bouwman, Frederick L., Jr., Graduate Assistant, Michigan State College.
Bullock, Jane Ann, Graduate Student, University of Oklahoma.
Burnett, Frances L., Cornell University.
Chaet, Alfred B., Student, University of Massachusetts.
Currier, Joanne E., Graduate Assistant in Zoology, Vassar College.
D'Ambrosio, Gloria, Student, Northwestern University.
Davis, Earle A., Jr., Graduate Student, University of Illinois.
Dawson, William A., Student, Swarthmore College.
Donnelly, Orville W., Graduate Assistant, Williams College.
Fienemann, Barbara J., Teaching Fellow, Smith College.
Fingerman, Milton, Graduate Assistant, Northwestern University.
Foster, Richard W., Assistant Curator of Mollusks, Harvard College.
Frost, Robert C., Graduate Student, Rice Institute.
Gerold, Nicolas J., Jr., Assistant, Cornell University.
Getman, Herbert C., Graduate Student, Brown University.
Gilmartin, Rosemary T., Graduate Student, Yale University.
Godfrey, Robert R., Graduate Assistant, New York University.
Goldstein, Lester, Graduate Student, University of Pennsylvania.
Grant, William C., Dartmouth College.
Grun, John, Student, Brooklyn College.
Hankins, Robert M., Research Assistant, University of Kansas.
Hansen, Bruce W., Student, Monmouth College.
Holle, Paul A., Graduate Student, University of Notre Dame.
James, Carolyn L., Vassar College.
Jones, Elizabeth J., Undergraduate, Oberlin College.
Kallen, Eugene J., Student, Columbia University.
Leonard, Barbara H., Instructor, Smith College.
Mabel, Judith B., Student, Goucher College.
Most, Sylvia N., Graduate Student, University of Pennsylvania.
Nanney, David, Research Fellow, Indiana University.
Patten, John A., New York University.
Peck, Harry D., Student, Wesleyan University.
Pulley, Thomas E., Assistant Professor, University of Houston.
Rieck, Alvin F., Marquette University.
Said, Rushdi, Student, Harvard University.
Schneiderman, Howard A., Fellow in Biology, Harvard University.
Sedar, Albert W., Student, State University of Iowa.
Sengbusch, Howard G., Graduate Assistant, New York University.
Sindermann, Carl J., University of Massachusetts.
Sippel, Theodore O., Laboratory Assistant in Zoology, Yale University.
Spiegel, Melvin, Graduate Student, University of Rochester.
Stafford, Nancy J., Teaching Fellow, Tufts College.
Telfer, William H., Graduate Student, Harvard University.
Tinker, Milton E., Teaching Assistant, University of Minnesota.
Weyl, Hilda, Student, Hunter College.
Williams, Austin B., Assistant Instructor, Kansas University.
Wilson, Katherine, Graduate Assistant, Washington University.

5. The Lalor Fellows, 1949

Albert W. Frenkel, University of Minnesota.
C. Albert Kind, University of Connecticut.
Albert I. Lansing, Washington University School of Medicine.
Jerre L. Noland, University of Wisconsin.
Arthur A. Woodward, Brown University.
Verner J. Wulff, University of Illinois.

6. The Atomic Energy Commission Fellows

Richard Abrams, University of Chicago.
Felix Friedberg, Howard University.
Marion Kayhart, University of Pennsylvania.
Jay S. Roth, Rutgers University.
Frank H. Rudenberg, Harvard University.

7. Tabular View of Attendance, 1945–1949

	1945	*1946*	*1947*	*1948*	*1949*
Investigators—Total	212	267	299	326	344
Independent	138	175	182	183	193
Under instruction	10	29	36	42	52
Library readers	38	38	36	50	55
Research Assistants	26	25	45		
Students—Total	96	122	131	123	128
Zoology	55	57	55	54	55
Embryology	23	30	33	29	31
Physiology	13	26	26	25	27
Botany	5	9	17	15	15
Total Attendance	308	389	430	449	472
Less persons registered as both students and investigators			2	6	2
			428	*443*	*470*
Institutions Represented—Total	*124*	*141*	*148*	*158*	*155*
By investigators	100	102	114	117	114
By students	49	56	56	68	68
Schools and Academies Represented					
By investigators	2	2	1	1	1
By students	2		1		
Foreign Institutions Represented					
By investigators	1	7	7	8	6
By students		5	3	4	3

8. Subscribing and Cooperating Institutions, 1949

Subscribing Institutions

Argonne National Laboratory
Boston University
George Washington University Medical School
Georgetown University Medical School
Indiana University
Institute for Cancer Research, Lankenau Hospital
Institute of Pennsylvania Hospital
Long Island Medical College
Marquette University
Morgan State College
National Research Council
National Research Council of Canada
Northwestern University
Ohio State University, Graduate School
Public Health Research Institute of New York City
Radcliffe College
Russell Sage College
Rutgers University
St. Louis University
Southwestern Medical College
Tulane University School of Medicine
U. S. Public Health Service
University of New Hampshire
University of Oklahoma
University of Pittsburgh
University of South Dakota
University of Wisconsin
Wellesley College

Cooperating Institutions

Amherst College
Brooklyn College
Brown University
Bryn Mawr College
Catholic University of America
College of Mt. St. Joseph-on-the-Ohio
College of Physicians and Surgeons
Columbia University
Cornell University
Duke University
Fordham University
Goucher College
Harvard University
Harvard University Medical School
Hunter College
Institute for Muscle Research
Johns Hopkins University
Johns Hopkins University Medical School
Eli Lilly and Company
Madison Foundation
Massachusetts Institute of Technology
Mount Holyoke College
Mount Sinai Hospital
National Cancer Institute
New York University College of Medicine
New York University, Heights
New York University, Washington Square College
North Carolina State College of Agriculture and Engineering
Oberlin College
Princeton University
Rockefeller Institute for Medical Research
Sloan-Kettering Institute
Smith College
State University of Iowa
Temple University
Tufts College Medical School
Union College
University of Chicago
University of Delaware
University of Illinois
University of Kansas
University of Maryland School of Medicine
University of Massachusetts
University of Michigan
University of Minnesota
University of Missouri
University of Pennsylvania Medical School
University of Pennsylvania
University of Vermont Medical School
University of Virginia
Vassar College
Washington University
Wesleyan University
Western Reserve University
Yale University

9. Evening Lectures

The Friday Evening Lectures, 1949

Friday, July 1
Dr. Albert Tyler ..... "Serological Aspects of Fertilization."
Friday, July 8
Dr. Hans Gaffron ..... "Problems in Photosynthesis."
Friday, July 15
Dr. J. H. Quastel ..... "Soil as a Medium for Biochemical Investigations."
Friday, July 22
Dr. Erik Zeuthen ..... "Recent Developments in the Cartesian Diver Technique, and Its Application to the Study of Respiration During Mitosis."
Friday, July 29
Dr. T. M. Sonneborn ..... "Environment, Cytoplasm, and Genes in the Control of Heredity in Paramoecium."
Friday, August 5
Dr. J. S. Fruton ..... "The Metabolism of Peptides."
Friday, August 12
Dr. F. A. Brown, Jr. ..... "The Mechanism of Color Changes in Crustacea."
Friday, August 19
Dr. M. E. Krahl ..... "Metabolic Activities of the Arbacia Egg."
Friday, August 26
Dr. S. Gross ..... "Experiments in Marine Fish Culture."

Other Lectures

Wednesday, June 15
Dr. Gunnar Thorson ..... "Reproductive and Larval Ecology of Marine Bottom Invertebrates."
Monday, July 18
Dr. Perihan Cambel ..... "Problems of Modern Turkey."
Wednesday, July 27
L. V. Foster ..... "Microscope Optics."
Wednesday, August 24
Dr. Howard A. Meyerhoff .....
Tuesday, August 30
Dr. Michael Graham ..... "Recent Marine Research in the Arctic."

10. Seminars, 1949

July 12
Anna R. Whiting ..... "Evidence that Response to Fractionation of X-ray Dose in Habrobracon Eggs is Cytoplasmic."
H. H. Plough, Madelon R. Grimm and M. L. Vogel ..... "Minus and Plus Mutations in Biochemical Requirements in *Salmonella typhimurium*."
P. W. Whiting and Marion E. Kayhart ..... "X-ray Mutations and Fecundity of Mormoniella."
July 19
Albert Frenkel ..... "The Effect of Ultra-Violet Light (2537 Å) on the Catalase Activity and on Photosynthesis of *Chlorella pyrenoidosa*."
Ernest Kun ..... "Biochemical Properties of Succinoxidase from *Salmonella aertrycke*."
Alfred Bliss ..... "Reversible Enzymic Reduction of Retinene to Vitamin A."
David Nachmansohn, S. Hestrin and H. Voripaieff ..... "An Enzymatic Product with Acetylcholine-like Activity, Derived from Brain Extracts."
July 26
Ethel Browne Harvey ..... "Growth and Metamorphosis of the Pluteus of *Arbacia punctulata*."
James A. Miller ..... "Effects of Temperature upon Survival of Newborn Guinea Pigs Subjected to Anoxia."
Alfred Marshak ..... "Evidence for Activity of Desoxy-ribonuclease in Nuclear Fusion and Mitosis by the Use of d-Usnic Acid."
Carl C. Speidel ..... "Motion Pictures Showing the Reactions of Cells in Frog Tadpoles to Implants of Tantalum."
August 2
Andrew G. Szent-Gyorgyi ..... "Investigations on Muscle Fibers."

E. L. Chambers and W. E. White ..... "The Accumulation of Orthophosphate and its Relation to Adenosine Triphosphate in the Fertilized Echinoderm Egg."
Abel Lajtha ..... "Energy Rich Phosphate in Nucleic Acids."
Elemer Mihalyi ..... "On the Structure of Fibrin Clots."
Brian A. Cookson ..... "Some Methods of Producing Traveling Contraction Nodes in Adult Frog Skeletal Muscle Fibers."

August 9

F. R. Hunter ..... "An Analysis of the Photoelectric Method of Measuring Permeability."
M. H. Jacobs, Carolyn M. Stout, Marion W. LeFevre and W. E. Love .. "How Simple Are the So-called Simple Hemolysins?"
Lois H. Love ..... "Hemolytic Action of Anionic Detergents."
A. K. Parpart and J. W. Green ..... "Potassium and Sodium Exchange in Rabbit Erythrocytes Treated with Butyl Alcohol."

August 16

Dorothy Wrinch ..... "Structure of Insulin."
J. M. Moulton ..... "The Development of Menidia-Fundulus Hybrids."
Ezra Allen ..... "Analysis of Degeneration in Primordial Germ Cells and a Hitherto Undescribed Germ Cell in Albino Male Rats."
C. G. Wilber ..... "Fat Metabolism in Arctic Fish as Compared with Temperate Form."

11. Members of the Corporation, 1949

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A CYTOLOGICAL AND CYTOCHEMICAL STUDY OF THE MALE ACCESSORY REPRODUCTIVE GLANDS IN THE JAPANESE BEETLE, POPILLIA JAPONICA NEWMAN

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Introduction

These studies were undertaken in order to investigate the cytological details of the accessory reproductive glands of Popillia, and to determine whether regional cytological differences could be correlated with differences in secretory activity. Some of the techniques of cytochemistry have been applied to these glands to elucidate the general nature of the secretion product, and to localize sites of origin of its components.

The male genital system of insects is typically characterized by the presence of a varying number of accessory glands appended to the principal effluent passages. These have been variously considered as analogous to Cowper's gland and the prostate of vertebrates (Hegetschweiler, 1820) and as seminal vesicles (Dufour, 1824). Escherich (1894) correctly described the functions and embryonic origins of the accessory glands of several Coleoptera. Those of Lucanus (a Scarabaeoid beetle related to Popillia) were shown to be of mesodermal origin. Escherich had demonstrated (1893) the similarities between the male genital tract of Lucanus and those of the Scarabaeidae. The conclusions of Escherich were substantiated by Blatter (1897) in a histological study of the accessory glands of Hydrophilus. Comparative studies of the male genital system in a large number of Coleoptera by Bordas (1900) elucidated the features of this system with its accessories and described the characteristic appearance of the accessory gland secretions. Studies by Rittershaus (1927) of the structure and biology of Anomala and Phyllopertha dealt briefly with the structure and function of the accessory glands of the male. The glands of these species, which are members of the same family (Rutelidae) as Popillia closely resemble those of Popillia. In these beetles the secretion product of the accessory glands serves as a vehicle for sperm transfer and coagulates in the female tract to form spermatophores.

Material and Methods

The entire reproductive tract was dissected from adult male Japanese beetles. The glands were carefully teased from their positions along the gut wall and allowed to fall into loose coils about the testicular masses. The systems thus removed were fixed and subsequently treated in a variety of ways, depending upon the particular structures or substances to be demonstrated. The specific techniques used will be summarized below in connection with the observations.

It is disadvantageous to handle the material as entire reproductive tracts because the coils of the gland become disposed at random about the remainder of the organs. Since in many of the techniques used it is advisable to employ adjacent slides as controls, it often happens that control and test slides contain sections of different regions of the gland. As a result, some levels have eluded treatment with some of the techniques. Indications will be given below of regions for which specific data are lacking.

Observations

A. General morphological considerations

Gross anatomy and relationships. There are two accessory reproductive glands in the adult male. They are long, coiled tubes which lie closely applied to the wall of the gut. Their length is approximately 4 times that of the abdomen, although, since they are extremely delicate, it is difficult to extend them fully. In the living animal the glands are a yellowish color. The small apical portion (diameter about 65 μ) tapers gradually to an expanded region (diameter about 246 μ) at the basal portion of the gland. The expanded sac-like structures continue posteriorly to the point at which the two glands join to receive the vasa deferentia and form the ejaculatory duct.

Microscopic anatomy. The following observations were made on material fixed in Flemming's strong solution, sectioned serially at 5 μ, and stained in iron-alum hematoxylin. Other glands fixed in Zenker-acetic were sectioned serially and stained with eosin-Y and methylene blue.

At its apical end, designated as Level I, each gland consists of an almost solid cylinder of moderately tall columnar cells 24 μ high and about 9 μ broad (Figs. 1, 16). Arranged radially, the cells rest upon a very thin basement membrane, and they bound a small, irregular lumen. In the lumen, a scanty, thin secretion lies upon the free ends of the cells. Each cell contains a large, ovoid nucleus near its basal end.

A short distance posteriorly, the diameter of the gland increases gradually to about 98 μ. The cells at this level (referred to as Level II) are of the same shape and size as those in Level I, but the diameter of the lumen has increased to approximately 60 μ (Fig. 7). The secretion in the lumen appears to differ little from that in Level I, but it is more abundant and contains numerous scattered small globules present in small numbers at Level I. The separation of the upper region of the gland into Levels I and II is largely a matter of convenience, based chiefly on differences in diameter.

About midway between the apex and the enlarged basal portion of the gland, the diameter increases to 158 μ and the lumen measures approximately 88 μ (Level III). The cells are much taller than those in the upper regions (Figs. 3, 22), measuring about 46 by 10 μ. Most of them appear to be carrying on intense secretory activity. That the secretory process is of the apocrine type is indicated by globules of secretion-packed cytoplasm which are budded or pinched off from the apices into the lumen. Levels II and III are united by a short intermediate zone in which Level II gradually blends into Level III (Fig. 8).

At this zone of transition two layers of striated muscle are found, for the first time, outside the basement membrane. The outer layer consists of narrow, separate,

parallel bands of longitudinal fibers; the inner, of an uninterrupted sheet of circular muscle. The total thickness of the muscle coats is 3 to 5 μ.

The accumulation of secretion in the lower regions of the gland lumen proceeds rapidly and exerts a marked influence upon the histology of the portion nearer the basal end. The increase in total diameter of the gland as it approaches the ejaculatory duct is accompanied by an increase in the diameter of the lumen and a decrease in the height of the epithelial cells. In the largest region of the gland (Level IV) the total diameter approximates 250 μ; the diameter of the lumen is 225 μ; the muscle coats remain 3 to 5 μ thick. The epithelial cells are cuboidal or squamous and measure about 10 μ in height. There is a gradual transition from Level III to Level IV. There is practically no evidence of secretory activity in cells typical of Level IV.

B. Special methods and observations

CYTOPLASMIC BASOPHILIA. Serial sections of material fixed in Zenker-acetic were stained with eosin-methylene blue. Adjacent sections were treated with ribonuclease [1] before staining.

The cells of all the levels of the gland show intense cytoplasmic basophilia. In Levels I and II, the cells have a dense perinuclear basophilic area from which radiate undulant basophilic strands. These fiber-like elements lie parallel to the longitudinal axis of the cell and pass around clear cytoplasmic areas above and below the nucleus (Fig. 10). The free secretion in the lumen consists of a uniformly acidophilic, granular mass of ground substance. In Level I it contains a few small, clear, unstained globules or vacuoles which are more numerous in Level II. Occasionally some parts of the ground-mass may stain with methylene blue, giving rise to a purplish rather than a blue tint (Fig. 7, edges of secretion mass).

The cells in Level III also show a perinuclear concentration of basophilia from which basophilic strands radiate toward the apices of the cells (Figs. 3, 8). The secretion droplets which are being shed from the apices of these cells are intensely and solidly basophilic. However, as these cytoplasmic globules break down and become parts of the free secretion mass their contents stain less intensely. The free secretion from this level down consists of an acidophilic granular ground substance which contains scattered clear globules (these two apparently produced in Levels I and II), and numerous somewhat basophilic larger elements produced in Level III.

The cells in the largest region of the gland show none of the streaming basophilic fibrillar structures characteristic of other levels. In these cells (Level IV), the nucleus is capped proximally and distally by strongly basophilic masses (Figs. 3, 4, 5). These cells show no evidence of secretory activity.

After treatment with ribonuclease all traces of cytoplasmic basophilia are removed. The strands or fibers remain as pale, acidophilic "ghosts" in the cytoplasm, although no remnants of the perinuclear concentrations persist (Figs. 2, 6, 11). The basophilic elements free in the lumen, however, are not affected by the enzyme (Figs. 4, 6).

Nuclear basophilia is virtually unchanged by ribonuclease, with the exception of the two or more large, conspicuous nucleoli which become partially acidophilic;

[1] Supplied by Dr. William Montagna of this laboratory.

these give the impression of a basophilic shell which encloses an inner acidophilic mass (cf. nucleoli in Figs. 5, 6). In untreated control sections the nucleoli are deeply basophilic.

GLYCOGEN AND OTHER POLYSACCHARIDES. Material fixed in cold Rossman's fluid and sectioned serially at 5 μ was treated according to the technique of Hotchkiss (1948). Sections exposed to saliva to digest glycogen were processed simultaneously as controls, and for the demonstration of polysaccharides other than glycogen.

Aggregations of Schiff-positive granules appear in the cells of all levels of the gland. In Levels I and II these masses lie both below and above the nucleus and extend partway from the nuclear membrane toward the apex of the cell. These aggregations evidently occupy the spaces between the basophilic strands described above, and they are more numerous in Level I than in Level II. These elements give a rather weak color reaction when compared with that in the glycogen-rich cells of the neighboring fat-body. The granular secretion in the lumen also gives a weak, pink reaction with leucofuchsin, but the small clear droplets are negative.

At Level III the tall apocrine cells show aggregates of pink granules at their free ends, and only a few near the nucleus. The granules are particularly concentrated in the terminal protoplasmic processes, and these small masses appear to recolorize the Schiff reagent more thoroughly as they break down and become the larger bodies in the free secretion. These bodies, which correspond to the basophilic components noted in eosin-methylene blue preparations, stain a deep magenta with leucofuchsin. (Fig. 9).

PLATE I

All figures are of paraffin-embedded material sectioned at 5 μ. Magnifications given are of the figures as they appear here.

FIGURE 1. Basophilia, Level I, eosin-methylene blue. Note cytoplasmic strands streaming from juxtanuclear concentrations; nucleoli. 750 ×.

FIGURE 2. Basophilia, Level I, eosin-methylene blue after ribonuclease digestion. Note persistence of basophilia in rims of nucleoli; acidophilic "ghosts" of cytoplasmic strands. 750 ×.

FIGURE 3. Basophilia, Level III (upper), Level IV (lower); eosin-methylene blue. Note deep basophilia in protoplasmic processes of Level III cells; basophilic elements in free secretion. 750 ×.

FIGURE 4. Basophilia, Level IV, eosin-methylene blue. Note basophilic areas surrounding nuclear caps; basophilic secretion bodies. 330 ×.

FIGURE 5. Same cells shown in Figure 4. 750 ×.

FIGURE 6. Basophilia, Level IV, eosin-methylene blue after ribonuclease digestion. Note disappearance of nuclear caps; persistence of basophilic rims of nucleoli and of basophilic elements in free secretion mass. 330 ×.

FIGURE 7. Basophilia, Level II. 330 ×.

FIGURE 8. Basophilia, transition zone Level II–III. Note first appearance of basophilic bodies in secretion mass.

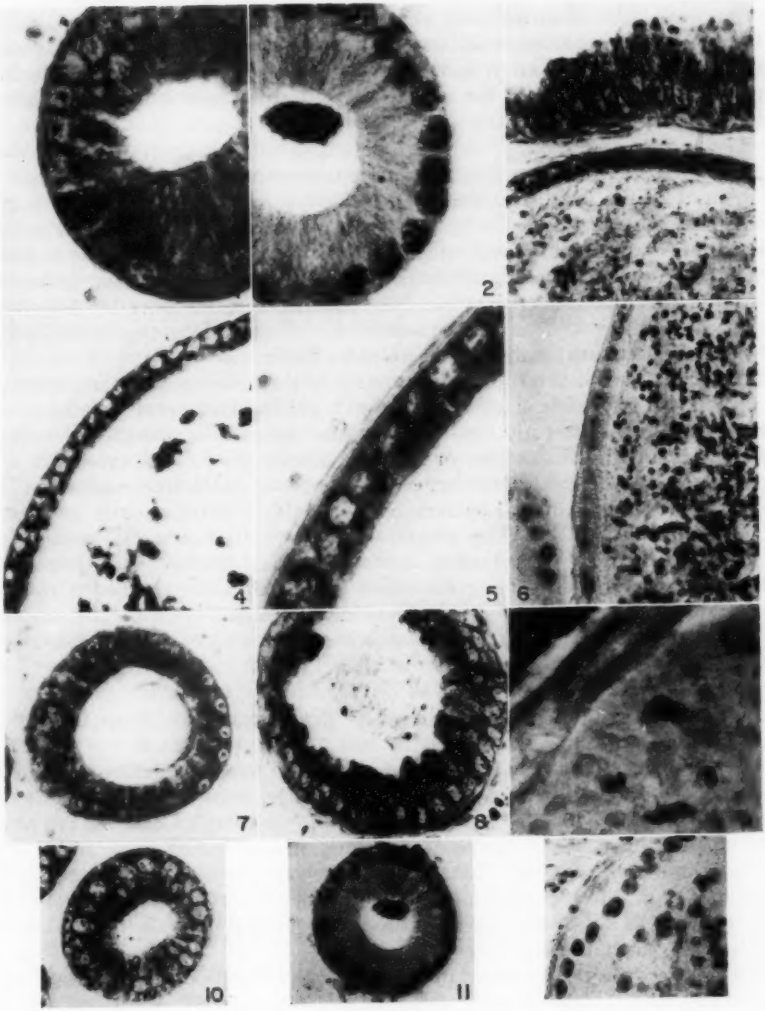
FIGURE 9. Periodic-acid-Schiff reaction, Level IV. Note glycogen bodies in cells; deeply-stained mucus flocs in secretion. Dark mass at upper left corner is glycogen in adjacent fat-body cells. 750 ×.

FIGURE 10. Basophilia, Level I–II; as in Figure 1. Note clear cytoplasmic areas. 330 ×.

FIGURE 11. Basophilia, Level I, after ribonuclease digestion. Same section as in Figure 2. 330 ×.

FIGURE 12. Toluidine blue reaction, Level IV. Note metachromatic flocs in secretion; ground-mass of secretion is greenish. 330 ×.

PLATE I



At the largest level of the gland the cuboidal cells contain few granules comparable with those in upper levels. Each cell shows, near its nucleus, a few moderate-sized masses which stain intensely, and a few smaller granules near the free border which stain less deeply (Fig. 9). The free secretion at this level does not differ from that seen at Level III.

Except for the moderate-sized masses in cells at Level IV, none of the elements described above is destroyed by salivary digestion prior to treatment with the Schiff reagent. The appearance of the secretion within the lumen is not visibly affected by salivary amylase.

METACHROMATIC SUBSTANCES. For the demonstration of metachromasia, material was fixed in basic lead acetate-formaldehyde, sectioned serially at 5 μ, and stained in 0.1 per cent toluidine blue in 1 per cent alcohol; these sections were dehydrated and washed in 95 per cent and absolute alcohol, and mounted in balsam. Parallel series were stained in alcoholic toluidine blue at pH 3.8 and in a similar solution of the stain buffered to pH 5.0. Control sections were incubated for 6 hours with hyaluronidase as recommended by Wislocki, Bunting, and Dempsey (1947) and then stained with toluidine blue as above.

In sections stained at pH 5.0, the secretion in the lumen remains unstained, and the cytoplasm of the cells at all levels shows a general faint metachromasia. This non-localized and apparently non-specific metachromasia is probably of no significance. In unbuffered solutions of toluidine blue at pH 3.8, the cytoplasm of the gland cells is basophilic, while the large bodies scattered in the free secretion at Level III and below (which, it will be recalled, are highly Schiff-positive) are strongly metachromatic (Fig. 12). The ground substance of the secretion mass stains a bright greenish-blue. The granules in the cytoplasm of the gland cells are not metachromatic; if they do stain green, like the ground-mass in the lumen, this color is masked by the basophilia of the surrounding cytoplasm.

Treatment with hyaluronidase has no observable effect on the metachromatic staining of these substances with toluidine blue.

LIPIDS. Several methods were used to demonstrate lipoid sustances in the accessory glands.

1. SUDAN BLACK: thin frozen sections of material fixed in calcium-formaldehyde and embedded in gelatin were treated with sudan black according to Baker's (1944) directions. Such treatment reveals, at all levels, a variety of discrete cytoplasmic elements. At Level I, dot-like and irregularly-shaped bodies lie scattered in the cytoplasm and gathered at the basal ends of the nuclei. Many of these blackened structures are of different sizes and form irregular rims about clear vacuoles (Fig. 13). In the cells in Level II the vacuoles are larger and generally more numerous, lying between the nucleus and the free border of each cell. At Level III (Fig. 14) these bodies are similar to those in Level II in distribution and quantity, and it is apparent that no sudanophilic elements are concentrated in the protoplasmic processes, although some vacuolate bodies lie near the free ends of the cells. In the lower regions of the gland the appearance of the blackened structures is similar to that in upper levels, but they are fewer in number and lie chiefly near the nuclei. Reaction of the free secretion in the lumen to sudan black treatment in-

dicates that small amounts of lipoid material are present at all levels; the secretion mass shows scattered small droplets of sudanophilic substance lying dispersed in the ground-mass. In Level II, rows of small blackened droplets appear just outside the free borders of the cells, indicating that Level II is probably the chief site of release of the lipoid elements in the secretion. However, lipoid droplets are present in the lumen at Level I also. These small sudanophilic droplets apparently correspond to the small clear globules which remain unstained by eosin-methylene blue and by the Schiff reagent.

2. SUDAN BLACK FOLLOWING POSTCHROMATION: material fixed in calcium-formaldehyde was treated with dichromate-calcium before embedded in gelatin, sectioned on the freezing microtome, and colored with sudan black and carmalum (Cain, 1947a).

This manipulation reveals much additional lipoid material in the gland cells. The cells near the apex of the gland contain massive clumps of sudanophilic material which occupy almost the entire volume of the cytoplasm, above and below the nucleus (Fig. 16). Cells in Level II are similarly rich in sudanophilic elements, but these become less numerous and more scattered in the cytoplasm at the transition zone between Levels II and III. In this zone, and in Level III, the majority of the sudanophilic elements are clearly vacuolated, and they lie chiefly in clumps above and below the nucleus (Figs. 17, 18). Level IV cells show small clumps of blackened vacuolated bodies near their nuclei.

The free secretion is more sudanophilic after postchromation; many additional small to moderate-sized lipid droplets, some in Level I but more numerous in Level II and below, are revealed (Figs. 16–18).

3. ACID HEMATEIN ROUTINE: phospholipines were demonstrated by Baker's (1946) acid hematein test. This technique gives results essentially similar to those obtained by the use of Cain's postchromed-sudan black method, except that in the cells below Level II fewer cytoplasmic structures are colored. The crowded clumps previously noted in cells at Level I and upper Level II stain a deep blue to blue-black with acid hematein (Fig. 15). Unfortunately, only sections of the upper and lower transition zones of Level III have been found in these preparations; in the former, scattered vacuolated structures showing some concentration toward the nucleus are in evidence. Near the beginning of Level IV, the cells show positively-staining perinuclear elements which extend toward the free border (Fig. 21). Cells typical of Level IV present, very clearly, concentrated small globular aggregates at the upper ends of their nuclei, and some globules below the nuclei.

The free secretion shows some small, blue-black globules at all levels; these are more numerous below Level I. Some of the larger elements of the secretion stain sporadically a dark blue, the ground-mass, generally brown to yellow (negative), and the remaining large hyaline areas (Fig. 21) do not stain at all.

4. ACID HEMATEIN FOLLOWING PYRIDINE EXTRACTION: this is the control procedure for the acid hematein test for phospholipine (Baker, 1946). In the accessory reproductive glands, none of the cytoplasmic acid hematein-positive elements are present after the application of the pyridine extraction test (cf. Fig. 19 with 15, 22 with 21). This, according to Baker (1946) and Cain (1947 b), identifies the cytoplasmic acid hematein-positive substances as phospholipine. It may thus be

concluded that all cytoplasmic bodies stained by acid hematein in this material contain phospholipine.

Comparison of the reaction of the free secretion to acid hematein before and after pyridine extraction reveals that some, but not all, of the reactivity of the globules is removed by the action of the solvent (cf. secretion mass, Figs. 21 and 22). This substantiates the result of treatment with sudan black, which showed that some lipid was present in the secretion product; the acid hematein test further demonstrates that at least some of the lipid in the secretion consists of phospholipine. Since considerable amounts of residual secretion stain with acid hematein even after exposure to pyridine, particularly in the moderate to large bodies, this bears out the other evidence that some of it is non-lipoid.

5. Osmication techniques: treatment of this material with such techniques as that of Worley (1946) reveals vacuolar and dot-like structures within the gland cells, more or less similar to those described after other methods. Some small to medium-sized secretion bodies in the lumen are intensely impregnated by this technique; in general, however, they do not correspond to the sudanophilic and acid hematein-positive bodies. The usual unreliable nature of osmication techniques makes it difficult to interpret the nature of these impregnated elements.

Acid-fast substances. The distribution of the weakly Schiff-positive granules at both ends of the nuclei in the secretory cells suggested the possibility that they might consist of pigments. Therefore, 5 μ paraffin serial sections of material fixed in Zenker-acetic were subjected to staining with Verhoeff's carbol-fuchsin methylene blue for the demonstration of acid-fast material (Lillie, 1948). The granular structures gave a weak, indefinitely pink reaction to this technique also; this cannot be considered a positive reaction as compared with the behavior of certain intensely acid-fast granules in neighboring fat-body cells.

Plate II

All figures are of gelatin-embedded material sectioned on the freezing microtome.

Figure 13. Sudanophilic structures, Level I; sudan black, carmalum. Note scattered globular and vacuolar bodies. 750 ×.

Figure 14. Sudanophilic structures, Level III; sudan black, carmalum. Note scattered vacuole-containing bodies between nucleus and free border. 750 ×.

Figure 15. Acid hematein, Level I. Note masses of formed bodies; scattered stained globules in secretion. 330 ×.

Figure 16. Sudanophilic structures, Level I; sudan black after postchromation. Cf. picture with Figures 13 and 15. 330 ×.

Figure 17. Sudanophilia after postchromation, upper end of transition zone, Level II–III. Cf. abundance and distribution of blackened bodies with that in Figure 16. Note sudanophilic elements in secretion mass. 330 ×.

Figure 18. Portion of same section as in Figure 17, 750 ×. Note concentration of Golgi vacuoles at nuclear caps, progression toward free border.

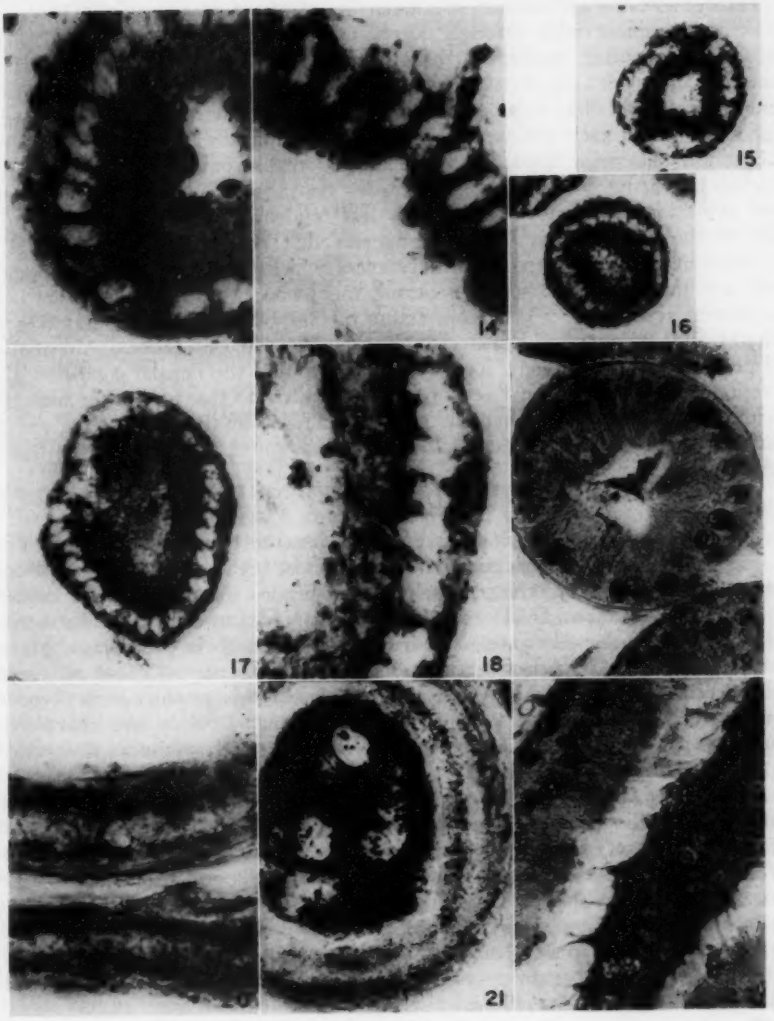
Figure 19. Acid hematein after pyridine extraction. Cf. Fig. 15. 750 ×.

Figure 20. Acid hematein, Level IV. Note aggregations of vacuole-containing Golgi-bodies at nuclear caps (cf. basophilic areas, Fig. 5). 750 ×.

Figure 21. Acid hematein, transition zone, Level III–IV. Note stained structures at nuclear caps, progression toward free border; deeply-stained bodies in free secretion; large clear areas in secretion mass. 330 ×.

Figure 22. Acid hematein after pyridine extraction, Level III. Cf. Figure 21 for removal of all cytoplasmic stained structures, loss of some staining capacity in free secretion. 330 ×.

PLATE II



It seems pertinent, at this time, to add a note concerning the appearance of the secretion product in the lumen after various fixatives. The variation in the appearance of the secretion mass following exposure to different fixatives makes it rather difficult to be certain of the correspondence of particular constituents, on the basis of size and appearance, in comparing results of various tests. In general, following treatment with strongly alcoholic fixatives (*e.g.*, Rossman, Zenker), the secretion product appears to consist of a finely-granular ground substance, somewhat shrunken away from the cells of its origin, containing scattered flocculent masses, some small clear vacuoles, and a few larger vacuolar structures. However, after exposure to such comparatively mild fixing fluids as calcium-formaldehyde, the ground substance appears as a mass of small, somewhat hyaline globular structures, bearing scattered larger globules or vacuoles of various sizes; under these conditions the mass shows much less tendency to shrink away from the walls of the gland. This behavior suggests that the ground material and the flocculent masses are composed of substances which tend to imbibe water and to swell, and that the milder fixatives permit this to occur to a certain extent before coagulation supervenes. The rapid action of the strongly alcoholic fixatives precipitates and coagulates the material before imbibition can occur. Alternatively, and perhaps more acceptably, it may be assumed that the normal condition of the secretion in the living animal is more or less similar to that seen after fixation in calcium-formaldehyde, and that this picture is altered by the dehydrating and precipitating action of the stronger fixatives and subsequent treatment of the material with alcoholic fluids.

Discussion

The observations of previous investigators on the histological features of the accessory glands indicate that some similarities exist between the glands of Popillia and those of other more or less closely related beetles. Bordas (1900) described the glands of Lucanus and of Dorcus (family Lucanidae) as composed of a secretory epithelium containing elongate, cylindrical cells, with their protoplasm granular near the base and compact and fibrillar near the free border. These cells release their secretion products by rupture, and globules of this product were frequently seen attached to the cell by a pedicel. Features such as these are characteristic also of the gland of Popillia, but only in the relatively restricted area designated in the present study as Level III.

Rittershaus (1927), working with two members of the same family as that containing Popillia (Anomala and Phyllopertha, fam. Rutelidae), briefly described differences in the type of epithelium present in different regions of their glands. She differentiated between tall, columnar epithelial cells and cuboidal to flattened cells. The lumen of the gland was described by this author as small in the upper region, filled with protoplasmic processes of the epithelial cells; in the lower region, the lumen was said to be clearly expanded and filled with glandular secretion. Rittershaus assumed that the protoplasmic processes were signs of strong secretory activity on the part of the cylindrical cells.

The observations outlined above indicate that regional histological differences in the gland, accompanied by differences in secretory activity, are characteristic also of Popillia. A detailed study of the glands in this species reveals, however, that there are more than the two regions described by Rittershaus. The morpho-

logical differences noted in this study include variations in the diameter of the gland and of its lumen, differences in shape, size, and type of cells, and differences in the amount and nature of the secretion product occupying the lumen. It is apparent that the secretory processes in Levels I and II differ somewhat in characteristics and in type of product from those in Levels III, and that Level IV, as indicated by Rittershaus in other species, serves mainly as an expanded reservoir for the collection and retention of secretions elaborated in the upper regions of the gland.

Cytoplasmic basophilia of the type exhibited in cells of the gland at all levels has been established by numerous investigators, in other materials, as indicating sites of ribonucleoproteins (Brachet, 1940, 1947; Dempsey and Wislocki, 1945, 1946). Intense cytoplasmic basophilia removable by ribonuclease has been reported as characteristic of cells secreting large amounts of protein (Brachet, 1940, and Caspersson *et al.*, 1941, for acinar cells of pancreas; Noback and Montagna, 1947, for salivary gland cells and acinar pancreas cells). In addition, Greenstein (1944) states that cells which synthesize large quantities of protein characteristically have large nucleoli. Noback and Montagna (1947) demonstrated that one or more large nucleoli are present in the nuclei of acinar cells of the pancreas and salivary glands, and that these nucleoli contain ribonucleoprotein. Among others, Brachet (1940) and Dempsey and Wislocki (1945) have also localized ribonucleic acid in cell nucleoli. The present studies have demonstrated the presence of ribonucleoprotein in the cytoplasm and in the large nucleoli of cells of the accessory reproductive glands; this may be considered as an indication that the secretion product of these glands is at least partially protein in nature.

The differences in the shape and distribution of cytoplasmic ribonucleoprotein structures in cells at different levels in the gland are presumably correlated with differences in the secretory activities of the different regions. For example, in the upper levels, where all techniques reveal active secretion, basophilic elements stream from the nuclear membrane to the free border of the cell. In Level IV, however, where cells are evidently not active in secretion, the cytoplasmic nucleoproteins are restricted to a limited and concentrated area surrounding the nuclear caps. These same areas in cells at Level IV also contain lipoid bodies which resemble the Golgi element (vide infra).

Failure to demonstrate appreciable amounts of stored glycogen in cells at any level except the lowest portion of the gland may also be taken as an indication that Level IV is not a secretory region. Other polysaccharides, Schiff-positive after periodic acid but not removed by salivary digestion, are present in accumulations between the nuclei and the free borders of actively secreting cells in the upper levels, but these are present in much smaller amounts in the cells of Level IV. These accumulations lie in cytoplasmic regions which show no affinity for methylene blue; they may be regarded, in view of the apparent polysaccharide nature of the released secretion (see below), as precursors of the secretory products of the cells in which they lie. No regional differences in their cytologic localization are apparent, except their paucity at Level IV.

It is difficult to determine the exact nature of the cytoplasmic structures colored by sudan black without post chromation. However, previous work in germ cells of Popillia has demonstrated that both mitochondria and Golgi-derivatives are re-

vealed by such treatment (unpublished results). The scattered dot-like and vacuole-containing structures shown in cells of the accessory gland by this technique may represent mitochondria and some dispersed and poorly-preserved constituents of the Golgi element. In tissues so treated the cells at any level do not show concentrated juxtanuclear groups of Golgi vacuoles. In thin frozen sections there is a progression of vacuolated sudanophilic structures from the nuclear membrane to the free border, and an apparent increase in size of the vacuoles as they approach the apex of the cell. These things may indicate that these elements are like Golgi-bodies, and they presumably participate actively in secretory processes. Except for their scattered disposition and fewer numbers, they resemble the vacuolated elements demonstrated by sudan black after postchromation; they are like the constituents of the Golgi element described by Cain (1947a) in secretory cells of the alimentary canal of the leech Glossiphonia.

The striking regional differences in the abundance and distribution of lipid elements demonstrated by sudan black after postchromation, and by the acid hematein technique, are conspicuously at variance with the results of simple treatment with sudan black. With these techniques the dense masses of clearly localized droplets and globules shown in Levels I and II have no counterpart in cells at Level III and below. The massive and densely-packed nature of these elements makes it impossible to ascertain the exact morphological nature of the individual bodies. It seems probable, however, that they represent the more or less complete cellular complement of mitochondria and Golgi-bodies, some of which are lost in preparation unless the material is treated with potassium dichromate. The disparity in concentration of these elements between cells in Levels I and II and those in the lower regions of the gland suggests that secretory activity is greater in the upper levels. The appearance of the cytoplasmic phospholipines in these densely-packed cells does not indicate the occurrence of lipophanerosis, as described by Cain (1948) in certain neurones of Helix. This phenomenon was ascribed to a breakdown and release in the cytoplasm of all the cellular phospholipines, resulting in a tendency of the cytoplasm to stain uniformly. In the present instance the cellular phospholipines are not generally distributed, but they are definitely and discretely localized.

The appearance of the free secretion mass indicates that its lipoid components are produced in Levels I and II; these elements first appear in the free secretion in the areas of the gland characterized by an abundance of cytoplasmic phospholipines. It has been noted, (a) that globules of sudanophilic material are found in the secretion in Levels I and II; (b) that sudanophilic droplets lie in a fringe along the borders of cells in Level II, suggesting their having been released at this level; and (c) that certain of the components of the free secretion mass contain phospholipine. The possibility then remains that some of the cellular phospholipine demonstrated in the upper regions of the gland represents a phospholipine-containing product of secretory activity in these cells.

In areas of the gland where cytoplasmic lipoid structures are not obscured by overcrowding, it is clear that the nuclear caps which stain intensely with acid hematein represent the Golgi element as demonstrated by sudan black in post-chromed preparations. Using the acid hematein technique, several authors have previously shown that the Golgi-bodies of a variety of tissues contain phospholipine

(Cain, 1947a, for alimentary epithelium of Glossiphonia, and 1948, for nerve cells of Helix; Thomas, 1947, also for nerve cells in Helix; Montagna, Noback, and Zak, 1948, for sebaceous gland cells of man). The present studies show that the Golgi element in cells of the accessory reproductive gland of Popillia also contain phospholipine, along with other lipoid components which cause the vacuolated bodies to color more deeply and more generally with sudan black than with acid hematein.

The presence of the Golgi element within localized cytoplasmic areas which are surrounded by intensely basophilic zones, together with Schiff-positive granules and possibly phospholipine secretion-precursors, suggests that the Golgi-bodies are involved in the elaboration or accumulation of these products.

With regard to the general question of the nature of the secretion product of these glands, it is apparent that it is a mixture of a number of substances, present in the secretion mass as discrete components. Certain of these elements are produced in, and apparently traceable to, particular regions of the long, tubular glands. Within the limits of accuracy of correlating the varying appearances of secretion bodies after various methods of fixation, the following synopsis of their characteristics seems warranted, on the basis of their reactions to the different techniques employed.

1. THE GROUND SUBSTANCE: usually acidophilic; pink reaction with leucofuchsin, not removed by salivary digestion; greenish stain with toluidine blue at pH 3.8, not removed by alcohol; negative reaction with acid hematein test; not sudanophilic. Produced in Levels I and II.

2. SCATTERED SMALL TO MEDIUM-SIZED GLOBULES: neither acidophilic nor basophilic; negative reaction to leucofuchsin; no stain with toluidine blue; sudanophilic; positive reaction (at least in part) with acid hematein, negative after pyridine extraction; osmiophilic? Evidently produced in Levels I and II.

3. SCATTERED LARGER FLOCS OR GLOBULES: basophilic, not affected by ribonuclease; intense purplish stain with leucofuchsin, not removed by salivary digestion; purplish metachromasia with toluidine blue at pH 3.8, unaffected by alcohol or hyaluronidase; sometimes stains with acid hematein, but if so, not removed by pyridine extraction. Produced by apocrine cells in Level III.

On the basis of the characteristics listed, the scattered masses of highly Schiff-positive and metachromatic material described in 3, above, may be interpreted as consisting of mucus. McManus (1946, 1948) has demonstrated that mucus stored in and released from goblet cells in the intestine gives an intense color with leucofuchsin after periodic acid treatment. Wislocki, Bunting, and Dempsey (1947) found that mucus from several sources, including submaxillary glands of the guinea pig, and synovial fluid of rhesus monkeys, gave a strong reaction with the Bauer test. The Wislocki group (1947) found also that mucus gave a characteristically strong purplish metachromasia with toluidine blue, sometimes, but not always (depending on its source) abolished by hyaluronidase. Hempelmann (1940) demonstrated that mucoproteins from various sources, differing chemically, could be distinguished from one another on the basis of metachromatic reactions with toluidine blue and polychrome methylene blue. The secretion of the apocrine cells of the reproductive gland, being metachromatic and Schiff-positive, has the general characteristics of the group of mucoproteins indefinitely termed "mucus."

Other reactions of this material are also significant; in tests of the acid hematein routine using pure substances, Baker (1946) found that mucus occasionally stained, but that the staining reaction was not removed by treatment with pyridine. This is also true of the secretion-masses in the present case, which sometimes give a positive reaction with acid hematein but retain their reactivity after pyridine extraction. In addition, mucus, owing to its acid content, should be expected to show basophilia not removable by ribonuclease; the bodies under discussion show this characteristic.

The granular nature of the intracellular precursors of this secretion product in Popillia, and the evident tendency of the substance to swell upon being released from the cell, are also generally characteristic of mucus. The failure of the precursor-granules to give reactions like those of the released substance is interesting. Baker (1946) in his account of the reactions of various compounds with acid hematein found that, as described above, mucus outside of cells quite often gave a positive reaction, while mucin in the goblet cells of the mouse intestine consistently reacted negatively. Conditions within cells are sufficiently different from those outside cells to make it unreasonable to expect substances to react identically in both situations. However, it would appear that the mucus in the accessory gland secretion differs from the mucus of human jejunal goblet cells, for example, shown by McManus (1948) to be strongly Schiff-positive both before and after its release.

The production of this component of the secretion mass has been ascribed to cells in Level III because it never appears in the lumen above Level III but is characteristically abundant at this level and below.

Since the ground sustance (1, above) which makes up the bulk of the secretion recolors leucofuchsin, it must be related to mucus. That it is not identical with mucus is evident from the fact that its reaction with leucofuchsin is not so intense, and that it never gives a typical purple metachromasia with toluidine blue, but stains green. In addition, it seldom, if ever, reacts with acid hematein, and it is acidophilic. A tendency to stain green with toluidine blue has been reported by Wislocki, Bunting, and Dempsey (1947) for striated muscle fibers, thyroid colloid, stratum lucidum and stratum corneum of epidermis, and occasionally for nuclei. The substance so stained in the present instance would seem to be related in no obvious way to any of the examples cited. One may suggest, on the basis of the appearance of this substance and its reaction with leucofuchsin, that it is related to the mucus produced in Level III. It is probable that both substances are mucoproteins, members of a miscellaneous and widely-distributed group of compounds characterized as protein-polysaccharide complexes differing from one another chiefly in the nature of the polysaccharide moiety and its substituents (Hempelmann, 1940; Meyer, 1945). The periodic-acid-Schiff reaction, as pointed out by McManus (1948), cannot be used to distinguish actual chemical differences within this group of compounds.

The protein nature of secretions in both categories (1 and 3) is inferred from the abundance and intracellular distribution of ribonucleoproteins in cells at Levels I, II, and III, and by the conspicuousness of the ribonucleoprotein-containing nucleoli in cells at these levels.

The third recognizable constituent (2, above) is characterized as either entirely phospholipine or a mixture of phospholipine and other lipoidal substances. The

site of its production has been indicated as Levels I and II; the characteristic droplets or globules of this product first appear in the lumen in these regions of the gland.

On the basis of the results obtained in this study, we may picture the secretion of the accessory reproductive glands as a viscous fluid, composed chiefly of different-sized globules of mucus and mucus-like polysaccharide-protein compounds, containing a certain amount of phospholipine and possibly other lipoids present as scattered droplets. Bordas (1900) described the secretion of these glands in the beetles Lucanus and Dorcus as homogeneous, compact, and "muqueuse," always gelatinous but coagulated by reagents into a hyaline mass. The secretion in Lucanus was described as showing (near the free borders of the cells) the presence of small, refringent globules, some adhering to cells and some free in the lumen. Rittershaus (1927) described and figured the appearance of the secretion mass in Anomala and Phyllopertha as consisting of a finely-granular ground substance containing larger clear bodies; the secretion was described as clumping together in the middle of the lumen and receiving additions of fresh secretion at its perifery.

A secretion product consisting of these components may be considered as admirably adapted to its several functions of lubrication, sperm-suspension, and spermatophore formation.

Summary

1. Differences in secretory activity have been correlated with morphological and cytological regional differences at successive levels in the accessory reproductive glands.

2. Intense cytoplasmic basophilia, abolished by ribonuclease, is characteristic of cells at all levels.

3. The Golgi element of the secretory cells is in the form of vacuolated spherules and is demonstrable by use of sudan black, particularly after postchromation. The Golgi element contains phospholipine.

4. The secretion product of the gland consists of a ground-mass of a mucus-like protein-polysaccharide compound, bearing scattered large globules of mucus and many smaller droplets of phospholipine, and possibly other lipoidal substances.

5. The mucus-like ground substance and the lipoid components are produced in the upper levels of the gland; the mucus globules originate in tall apocrine cells in the middle region; the lower levels apparently do not contribute to the secretion mass but serve as expanded reservoirs for the retention of the finished secretion product.

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STARVATION STUDIES WITH THE PARASITIC WASP HABROBRACON

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Introduction

The present investigation on the braconid known in genetic literature as *Habrobracon juglandis* (Ashmead) suggested itself as a test of the hypothesis that the sex difference in fat cell size (Grosch, 1948c) may be interpreted on the basis of single versus double storage of reserves. That is, females are suspected to possess, in the form of ovaries, an additional and dominant storage tissue, competing in activity and mass for space in the confines of the wasp abdomen.

Furthermore if utilization of eggs occurs similar to that described for other Hymenoptera (Flanders, 1938, 1942a, 1942b) it is desirable to determine when the process begins and to what extent it proceeds, because basic techniques of important investigations on the effects of irradiation involve Habrobracon egg production (A. R. Whiting, 1949; G. A. Heidenthal, 1945).

Finally, as reference for proposed investigations on morphogenesis, it was desirable to know the size relationships of major internal structures under the normal and the most extreme conditions of the laboratory environment.

Part I: Investigations at Incubator Temperature

Because a majority of experimental rearing of Habrobracon is conducted under incubation, attention was especially directed to this environment.

Materials and methods. Wild type stock 33 provided the normal animal types used, namely impaternate males and biparental females. Since the purpose was to investigate the organism under routine laboratory conditions, no exceptional provisions for humidity control were made. Open pans ($10 \times 8 \times 2$ in.) of water were (and are) maintained on the lowest shelf of the $28 \times 16 \times 28$ in. incubator. Temperature was maintained at 30–35° C. with maximum-minimum thermometer keeping the record. Shell vials (70×20 mm.), plugged with cotton (enclosed in cheesecloth), were the containers employed in stock maintainance and for all experiments. Daily examinations were made of all vials. Thus day of emergence from cocoon and day of death were obtained.

Since not even water was given to the animals under investigation, the "most extreme" type of routine laboratory environment was provided. Incidentally, results in a small scale exploratory experiment had shown average mortality to occur slightly earlier in groups provided with water, which probably is explainable by a tendancy of weakened individuals to blunder into the water and drown. This observation is consistent with Genieys' (1925) statement that mortality is very great when starving braconids are given nothing besides pure water.

For purposes of estimating the site of the major loss of substance during starvation, wasps were transected at the petiole and the weights of abdomens and weights of anterior regions were compared in freshly eclosed and in starved wasps.

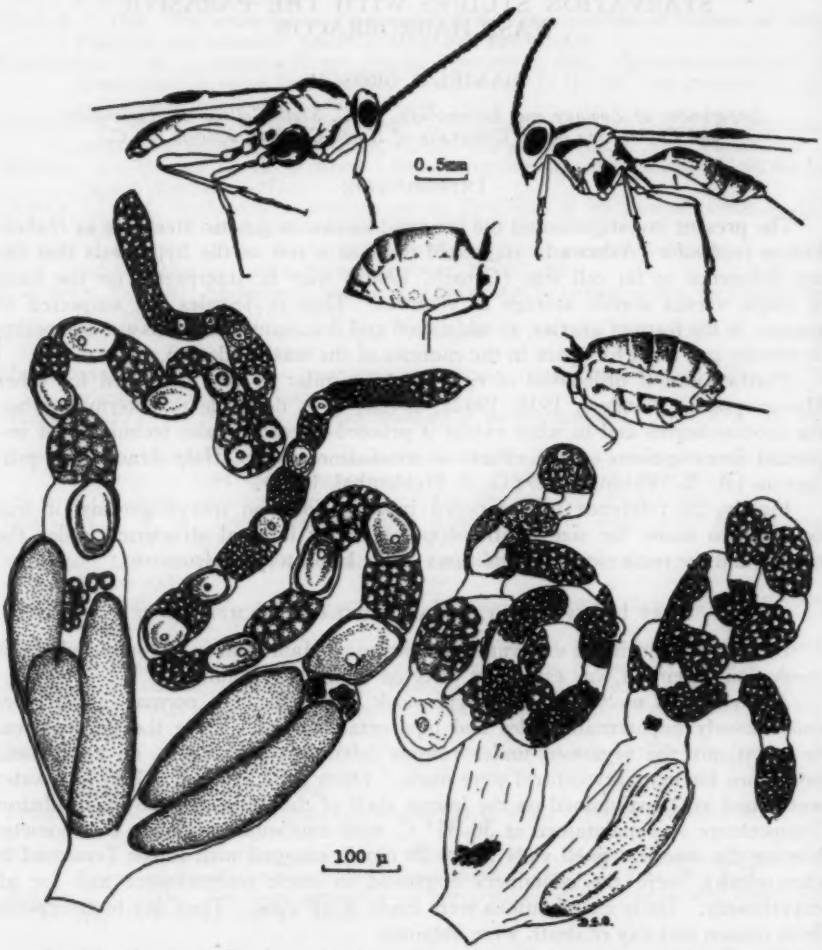

FIGURE 1. Above: Lateral views of a starved male (Left) and a starved female (Right) with abdomens of an unstarved male and female for comparison. The extreme dorsoventral flattening depicted can be seen in starved animals which are still quite active. It is interesting to note how this condition approximates the appearance of pinned museum specimens. Below: Ovarioles, each pair of which constitutes one of the two ovaries of (Left) an unstarved female recently emerged from her cocoon and (Right) a starved female dissected in what would have been the last or second last day of her life. The empty areas in the latter represent locations from which developing ova have disappeared.

For structural measurements wasps in various stages of starvation were dissected under a binocular microscope and the internal organs immediately fixed with 10 per cent formalin. This procedure was adopted because of the contrasts the fixative quickly develops in the ovaries and accessories. The preparation was then examined under a compound microscope and measurements of tissue elements taken by means of an ocular micrometer. The series of observations was continued until data was available for at least 5 wasps of each sex for each day during the starvation period.

Results

Longevity. The survival time in days as given by the mean and its standard error was $6.13 \pm .16$ for 100 males and $6.68 \pm .30$ for 100 females. All animals comprising these samples were within the normal size range and no correlation between the size of animal and the survival time in days was apparent (correlation constant $r = 0$). Measured from the time of eclosion the earliest deaths occurred on day 3, oldest survivors expired on day 10.

Weight loss. Comparisons of transected wasps indicate that weight is lost chiefly from the abdomen. For 10 males of selected size (2.35 to 2.47 mm. long) the anterior parts comprising the head and thorax-with-appendages averaged (mean with its standard error) $.47 \pm .04$ mg. at eclosion, and $.45 \pm .04$ after starvation; while the abdomens averaged $.52 \pm .05$ mg. at eclosion and $.38 \pm .06$ mg. after starvation. For 10 equivalent females, anterior parts averaged $.50 \pm .06$ mg. at eclosion and $.50 \pm .05$ mg. after starvation, while abdomens averaged $.82 \pm .07$ mg. at eclosion and $.38 \pm .06$ mg. after starvation. Weight losses from the abdomen are accompanied by dorso-ventral flattening which becomes extreme in the last two days of life (see Fig. 1). In fact, in this dimension the male abdomen becomes so thin it actually approaches translucence, with shadowed areas indicating the positions of the digestive tract and gonads.

Histological changes. Presented in Table I are measurements of internal structural units in unstarved (0 hour, 0 day; at eclosion) and starved wasps (unable to stand; therefore in the last day of life). Ten animals from each class are represented by this summarization. A summary of the series of examinations of internal structures made at daily intervals is untabulated because it merely provides values intermediate between the extremes of Table I. It is evident that the perceptible changes accompanying weight loss occur in the abdominal contents and especially in the "fat" cells. When the animal is adult this type of cell is found only in the abdomen (Grosch, 1949). In reference to the fat cells there is an additional untabulated observation on the abdominal contents of starved animals. That is, it is difficult to find 100 fat cells for measurement after starvation in contrast to conditions in unstarved wasps where there are typically several hundred fat cells. On the other hand there seems to be no significant change in number of urate cells as shown by the following means and standard errors: for unstarved males 35.20 ± 4.10, for starved males 31.25 ± 4.25, for unstarved females $34.33 \pm .27$, for starved females 31.16 ± 3.50.

Conspicuously absent in Table I are measurements of the ovarioles. This is due to the fact that because of contortion in starved animals, length could not be determined. However, diameters, as shown in the accompanying drawing (Fig. 1), change but little in ovarioles compared from starved and unstarved females. The

change which is very apparent in comparisons is the effect of a sorptive process. As seen, this has involved not only mature eggs but also developing eggs. Following these events in daily dissection, it is during the fourth day at incubator

TABLE I

Means and standard errors to represent dimensions in microns of internal structures of wasps 2.35 mm. to 2.47 mm. in length

Abdominal contents	Males		Females	
	Unstarved	Starved	Unstarved	Starved
Urate cell diameter	161.15± 2.64	110.22± 2.64	128.48±2 1.98	105.49± 3.19
Fat cell diameter	141.02± 1.87	89.10± 1.43	132.33± 1.43	78.21± 1.32
Crop				
Length	728.42±27.17	725.78±32.45	715.00±111.65	1005.40±119.02
Width	308.00±23.98	297.00±23.87	244.75± 44.11	294.80± 34.76
Stomach				
Length	889.13±14.41	880.00± 2.86	1031.25± 72.82	1086.25± 83.38
Width	330.00±27.50	253.00±14.74	481.25± 28.49	379.50± 33.99
Hindgut				
Length	891.00±33.60	861.63± 9.46	916.63± 15.18	941.75± 24.86
Width*	180.40± 9.57	190.63±15.62	231.00± 28.82	195.25± 4.95
Malpigh. tube				
Length	1353.00±60.39	1251.25±22.88	1375.00± 47.74	1320.00± 33.66
Width	33.00± 5.00	33.00± 1.65	33.00± 5.30	31.79± 8.80
Testis				
Length	270.60± 5.17	214.50± 4.18		
Width	184.80± 2.75	144.00± 1.75		
Accessory tube				
Length	371.25±22.77	368.50±26.07		
Width	74.25± 4.29	79.75± 4.29		
Abdominal ganglion (2nd last)				
Length	49.00± .55	49.00± .55	89.10± .55	93.50± 7.38
Width	49.00± .55	49.00± .55	91.63± 1.65	93.50± 8.69
Poison gland				
Length			770.00± 44.66	715.00± 27.50
Width			90.75± 8.36	107.25± 10.23
Reservoir				
Length			291.50± 11.88	253.00± 13.64
Width			17.05± .28	13.75± 7.26
Thoracic muscles diameters				
Dorsal long.	94.60± 3.85	96.80± 4.73	101.20± 4.73	96.80± 4.84
Alars	60.94± 1.54	59.40± .77	53.13± 1.54	50.95± 1.54
Promotors and remotors	25.19± .22	22.00± 2.09	22.00± 1.87	20.13± 1.65

* This dimension is given for the broadest part, the narrowest part in both males and females is about 77 μ in diameter.

temperature that the egg contents of the ovarioles are visibly depleted. To day 3 inclusive, a total of 10 to 12 full sized eggs can be found for the four egg sacs. The contents of both the mature and the developing ova appear to be quantitatively adequate during this period. After the third day changes are visibly apparent,

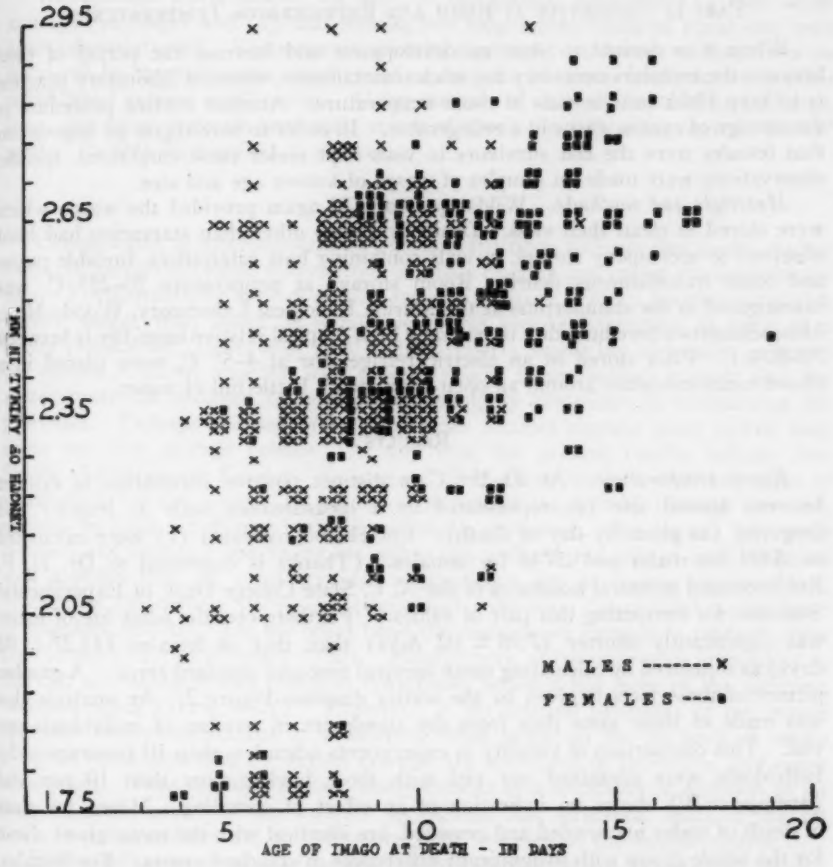

FIGURE 2. Scatter diagram showing the relative longevity of starved males and females of the sizes obtained in routine culture of Habrobracon. Notable is the tendency of females to live longer than males of equal size as reflected in the propensity of the squares to lie more to the right than the x marks.

especially in lessened opacity of egg regions. After five days of starvation no more than two eggs have been found in any dissected abdomen and after six days no full sized eggs have been found. On the other hand, there are no visible signs of sorption of developing eggs until a day or two before death (day 8 or 9 at incubator temperature). Incidentally, the relatively slight but definite change in testis size (Table I) also is not demonstrable until the last two days before death. In general these oösorption events have striking similarities to the observations on the honey bee and ants mentioned by Flanders (1942b) as being made first by Weyer.

Part II: Longevity at Room and Refrigerator Temperatures

When it is desired to slow up development and increase the period of time between the transfers necessary for stock maintainance, common laboratory practice is to keep Habrobracon vials at room temperature. Another routine procedure is the storage of excess wasps in a refrigerator. In order to investigate an impression that females were the last survivors in vials kept under these conditions, specific observations were made on samples of wasps of known age and size.

Materials and methods. Wild type stock 33 again provided the wasps which were stored in clean shell vials. Death by causes other than starvation had been observed to accompany storage in vials containing host caterpillars, inviable pupae and other miscellaneous debris. Room storage at temperature 20–25° C. was investigated in the summertime at the Marine Biological Laboratory, Woods Hole, Massachusetts where humidity is generally high (typical relative humidity is between 70–80%). Vials stored in an electric refrigerator at 4–5° C. were placed in a closed metal container around an open wide-mouth bottle full of water.

Results

Room temperature. At 20–25° C. a distinct positive correlation is evident between animal size (as represented by a measurement such as length) and longevity (as given by day of death). Correlation constants (r) were calculated as .3404 for males and .5746 for females. (Thanks is expressed to Dr. H. F. Robinson and technical assistants of the N. C. State College Dept. of Experimental Statistics for computing this pair of values.) Furthermore, the adult life of males was significantly shorter ($7.98 \pm .02$ days) than that of females ($11.27 \pm .02$ days) as measured by calculating mean survival time and standard error. A graphic picture of these facts is given by the scatter diagram Figure 2. An analysis also was made of these same data from the standpoint of number of individuals per vial. This comparison of viability in experiments when less than 10 (average = 5) individuals were contained per vial with those having more than 10 per vial (average = 20) shows no indication of an effect of crowding. Means for time of death of males uncrowded and crowded, are identical with the mean given above for the whole group with insignificant differences in standard errors. For females, mean survival time, in days with standard error $10.53 \pm .50$, was actually shorter in uncrowded vials as compared with the same computations in the larger groups, $11.63 \pm .16$. Differences between these means are not significant and the higher value for larger groups of females can be explained by chance inclusion of more of the longer lived wasps in the larger sample. It should be noted that even the trend is not what might be expected if adult population density is important in influencing the duration of life.

A second series of experiments at room temperature was performed with 100 females and 130 males of normal size kept in separate storage (10 per vial) from the time of their emergence as adults. No increased longevity was obtained for segregated females whether considered as a group or on the basis of extreme individuals. For segregated males a mean survival time in days of 8.38 ($s_{\bar{x}} = \pm .26$) indicates greater longevity.

Refrigerator temperature. At 4–5° C. the general principles (1) that larger animals live longer and (2) that females live longer than males of equal size were again demonstrated. Existence is greatly prolonged at lower temperatures as indicated by starved males which lived as long as 15 weeks and starved females which lived as long as 20 weeks. Using animals of normal size, 2.05 to 2.75 mm. long, the mean survival time with standard error for 193 males was 8.16 ± .17 weeks; for 109 females 9.71 ± .39 weeks.

Discussion

Comparisons of measurements of internal structures indicate that under starvation conditions the female differs from the male only by one major change, oösorption. Furthermore, in spite of smaller fat cells (Grosch, 1948c) females are shown to live longer than males of equal size. This fact is interesting in view of a recent publication (Georgiana, 1950) which reports longer life of females in honey fed culture. Taken together with present results these findings suggest that the stored materials obtained by oösorption are highly efficacious in maintaining life processes. Perhaps because of egg substance females contain more stored food *value* per unit storage volume. At any rate, the present results indicate that female abdomens are heavier. In a sense these views imply a different basis of metabolism for the sexes. In normal environment the female, through her diet, appears to have a different basis for metabolic processes. She ingests host body contents and although able to lay her oldest egg without this type of meal, it seems well founded that she must continue a caterpillar diet to insure continued egg production (Henschen, 1929) and circumvent oösorption (postulated on the basis of Flanders, 1942b). On the other hand the male shows interest in no food other than honey or syrup (Grosch, 1950). Furthermore, correlative with differences in the diet are demonstrable chemical differences between the feces of the different braconid sexes (Hase, 1922). Therefore, if the internal processes of the female normally involve substances in addition to those stored in the fat body, it seems a logical conclusion that the action systems are continued in starvation and cause oösorption. Thus revealed is mass disappearance of a substance in addition to fat body in confirmation of the authors' hypothesis that structural units of the fat body do not constitute the only depository for reserves in the female system.

While difference in type of metabolism is advanced as the explanation for differences in longevity between the sexes, difference in rate of metabolism is suggested in explanation of the correlation discovered between size and longevity within a sex. It is well known that smaller animals have a higher respiratory rate and that this rate is an index of metabolic activity (Heilbrunn, 1943).

Intimately associated with size differences in Habrobracon are differences in the size of microscopic structural units including fat cells and differences in the number of eggs produced (Grosch, 1948a). These situations are traceable to conditions during the larval feeding stage, the only period in the holometabolous life cycle during which the size increases (termed growth) occur. On this basis, crowding would be more likely to affect individual longevity if it occurred during the larval stage than at any other time during the Habrobracon life cycle. Furthermore, in contrast to the behavior of Drosophila, on which classic experiments in relation of population density to longevity have been made (see Hammond, 1938,

1939), stored adult Habrobracon adopt relatively quiescent attitudes in an undisturbed vial. The same pose and position may be maintained for hours or even weeks, depending upon the temperature. These facts could explain why results in an analysis based on group size demonstrated no noticeable effect of crowding on adult longevity.

An indication that segregated males live longer than males stored with females can be explained by the fact that mating preliminaries and the act of copulation involve excited active movements by the male (Grosch, 1948b). In the absence of mating stimuli these energy expending activities would not be indulged.

The bearing of the present results upon methods of investigation involving egg production and those pertaining to morphogenesis can be summed up quite briefly. The unfed adult should be employed in experiment or examined for phenotype during its first 48 hours, an adequate period of safety unless fat cells are under consideration. In the latter event examination should be made immediately upon emergence from cocoon as emphasized in a previous publication (Grosch, 1948c). Concerning comparisons of structural size, if genetic variation is under consideration, analysis should be made within sex types because Table I indicates slight sex differences in internal abdominal structures.

Studies on changes in microscopic anatomy are not typical of past trends in entomological literature. Starvation in adult insects has been approached chiefly from the standpoint of comparative longevity of unrelated species (Jackson, 1925; Maluf, 1939; Brues, 1946) or gross physiology of the intact organism (Uvarov, 1928; Wigglesworth, 1947). On the other hand, the amount of microscopic and histochemical observation made available through malnutrition and inanition experiments with vertebrates is impressively large, as indicated by consultation of almost any volume of Biological Abstracts. However, in histological detail, situations do not seem comparable and therefore literature on vertebrate changes are not reviewed herein.

Summary

1. In conditions of complete starvation, Habrobracon females live longer than males. This fact is more obvious at lower temperatures of storage.

2. There is a distinct positive correlation between size of a wasp and longevity. This is interpreted to have a basis in a lower metabolic rate in the larger organisms.

3. Weight loss is chiefly from the abdominal region which in the ultimate condition is extremely flattened dorsoventrally.

4. Histologically the greatest loss of material from both males and females is in number and size of the "fat" cells of the fat body. In females an additional loss occurs from the ovary, especially during the last stages of starvation. In wasps starved to the point of death the loss includes all the developing, as well as the mature eggs. Thus identified for the female seem to be two sources of reserve food supply. This point and attendant features are discussed from the standpoint of sex differences in structure, function and longevity.

5. Provided examination is made within the first two days, internal tissue other than the fat body should be in adequate quantitative state regardless of conditions of culture during the adult stage. It is also indicated that investigations employing ova should be started before two days after emergence from cocoons have elapsed.

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A TWO-TENTACLED, COMMENSAL HYDROID FROM CALIFORNIA (LIMNOMEDUSAE, PROBOSCIDACTYLA)

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Introduction

The new hydroid, *Proboscidactyla sp.*, which is the subject of this paper, was found living upon the tubes of a sabellid polychaete, *Pseudopotamilla ocellata* Moore, at Pacific Grove, California. The authors were able to collect and observe colonies during the summer of 1949 at the Hopkins Marine Station, and living colonies secured in September of that year were brought to Berkeley, California, for photography and further study.

The genus Proboscidactyla was established by Brandt (1834) for a new medusa, *P. flavicirrata*. Somewhat later Forbes (1846) described another medusa, *Willsia stellata*, for which he established the family Willsiadae. The generic name Willsia was changed to Willia by A. Agassiz (1862) because of an orthographical error on Forbes' part, and the family name then became Williidae. This family later came to include both Willia and Proboscidactyla. The Williidae were monographed by Browne (1904), who included six species of Proboscidactyla and two of Willia.

One of the most recent considerations of the family is that of Kramp (1940), who reviews the history of the Williidae and places this family with the families of Olindiidae and Moerisiidae in the suborder Limnomedusae, a group equal in rank to the Anthomedusae and Leptomedusae. Kramp defines the Limnomedusae as follows (p. 506):

"Hydrozoa with alternation of generations. The asexual generation is a sessile polyp with ability of asexual reproduction, with or without tentacles; the endoderm of the tentacles, when present, is in direct connection with that of the gastral cavity. The sexual generation is a velar medusa with hollow tentacles; gonads either on the walls of the stomach with perradial continuations along the radial canals, or on the radial canals alone; in the latter the umbrella-margin is provided with internal statocysts with an endodermal axis."

The suborder Limnomedusae was established by Kramp to include certain rather aberrant hydromedusans. This group seems to stem from both the Anthomedusae and Leptomedusae and may be polyphyletic.

Kramp (1940) also defines the family Williidae as follows (p. 509):

"Limnomedusae without statocysts; stomach with perradial lobes extending along the proximal parts of the radial canals; gonads surrounding the stomach and its lobes; radial canals branched; tentacular bulbs without ocelli."

The two genera of the Williidae were initially separated on the basis of the number of primary radial canals; Willia having six and Proboscidactyla four.

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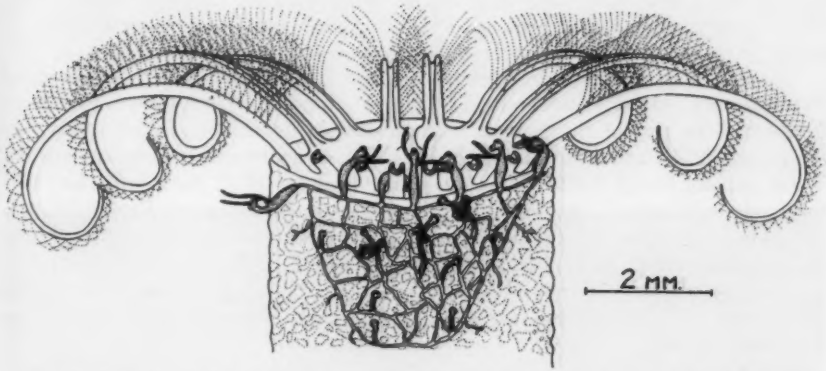
Some interesting information on the relationship of the above two genera was brought forth in a study of *Willia mutabilis* by Browne and Kramp (1939). In studying over 100 specimens they found 22 of these medusae with six primary radial canals and 41 with eight apparently primary radial canals. In the latter group, however, it was found that of the eight canals only four were truly primary, the other four arising not directly from the stomach but as branches of the primary canals, and thus being secondary in origin. Browne and Kramp also found specimens with 5, 7, 9, 10 and 11 apparently primary radial canals. The above data were taken by Browne and Kramp to mean that "The limit between the two genera Willia and Proboscidactyla is not so sharp as formerly supposed . . ." (p. 310), and that the species under study ". . . even tends to efface the limits of the two genera" (p. 302).

Until 1941 only two hydroids had been associated with the Williidae, and only in one case (*Willia stellata*) had the hydroid been shown to give rise to a specific



TEXT FIGURE 1. A colony of *Proboscidactyla* sp. in place upon a tube of *Pseudopotamilla ocellata*. Several of the worm's tentacles have been cut away, and the number of tentacles shown on the worm is reduced from the natural condition.

medusa of this family. This latter hydroid was first described as *Lar sabellarum* by Gosse (1857), who found it growing upon the tube of *Sabella vesiculosa* which he was keeping in his aquarium. According to E. T. Browne in "Plymouth Marine Fauna," 1931, Lar occurs on *Potamilla torelli*.

The generic name Lar was derived from the Roman "Lares" (household gods), and is particularly apt when one considers the oddly human appearance of this hydroid. Gosse noted that this hydroid had ". . . a most ludicrously-close resemblance to the human figure," and described its activities as follows (p. 113): "The head lobe moved to and fro on the neck; the body swayed from side to side, but still more vigorously backward and forward, frequently bending into an arch in either direction; while the long arms were widely expanded, tossing widely upward, and then waved downward, as if to mimic the actions of the most tumultuous human passion." Plate I, Figure 1 reproduces Gosse's figure of a colony of Lar

upon the extremity of a tube of *Sabella vesiculosa*; as will be seeen later, this hydroid closely resembles the form to be described in this paper.

The production of medusae by the hydroid *Lar sabellarum* was described by Hincks (1872), who noted that the medusae produced were similar to *Willia stellata*. Browne (1896), in a thorough study of the growth of *Willia stellata*, was able to show clearly that the medusa of *Lar sabellarum* actually was *Willia stellata* and so *Lar sabellarum* became a synonym of *Willia stellata*. The family Laridae, which had been established for the hydroid Lar was thus invalidated, although Fraser (1918, 1946) continued to use Gosse's Lar and Laridae.

The second known hydroid of the Williidae was described by Mereschkowsky (1877) as *Monobrachium parasitum*, for which he created a new family, Monobrachiidae. This hydroid differs from Lar in that it possesses but one tentacle, produces medusae with four primary radial canals and is found around the siphons on the shells of small subtidal bivalved molluscs. This hydroid is thought to be a Proboscidactyla because of the branching radial canals of its medusa, although it has not been associated with any specific adult medusa. Again Fraser (1918, 1946) recognizes the family name Monobrachiidae rather than the Williidae, of which he makes no mention.

In 1941, Uchida and Okuda described a new "Lar" which they found living on *Potamilla myriops* in Akkeshi Bay, Hokkaido, Japan. This hydroid was shown by them to be that of the medusa *Proboscidactyla flavicirrata*. This meant that the genera Willia and Proboscidactyla were both characterized by similar hydroids. These authors found, further, that the young medusae of *P. flavicirrata* had four, five or six radial canals, spanning the difference between Willia and Proboscidactyla. On the basis of the above evidence, plus the data of Browne and Kramp (1939) on the variability of *Willia mutabilis*, Uchida and Okuda united the two genera, adopting the name Proboscidactyla because of priority. Although not stated by Uchida and Okuda, the family name should accordingly be changed from Williidae to Proboscidactylidae.

Description

a. *Habitat*

The new Californian hydroid is found growing upon the tubes of a sabellid polychaete, *Pseudopotamilla ocellata* Moore, and has been collected from several stations along the Monterey Penninsula from Carmel Cove to Point Pinos. The sabellid seems to be restricted to rocky areas of the intertidal zone around the mean lower low water line, where it lives in crevices and under or between rocks. The tube of the worm is of a parchment-like substance, richly embedded with sand grains, although the terminal free portion is frequently less sandy. The sabellid is free to turn within its tube. When the worm withdraws, the terminal portion

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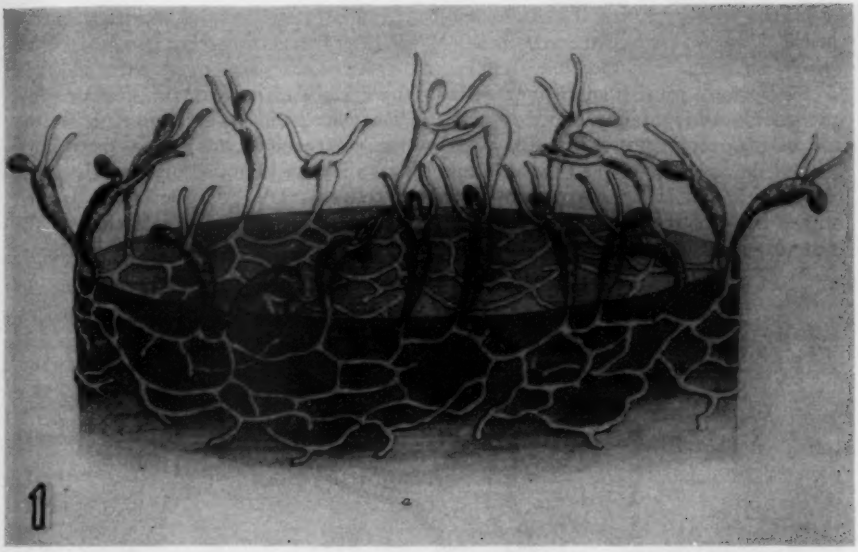
Plate I

Figure 1. A reproduction of Gosse's (1857) figure of *Lar sabellarum* on the extremity of a tube of *Sabella vesiculosa*.

Figure 2. A living colony of *Proboscidactyla* sp. on a tube of *Pseudopotamilla ocellata* (ca. 25 ×).

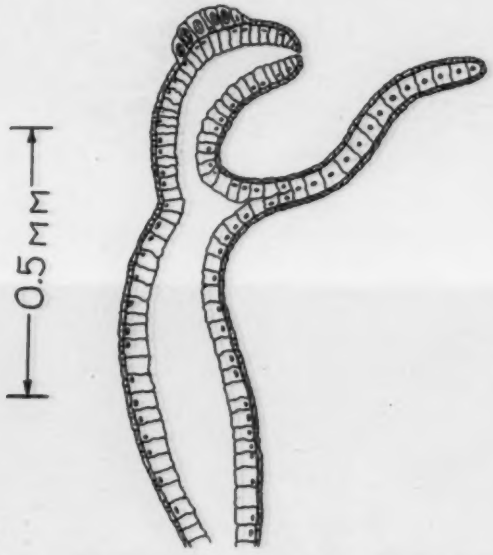
PLATE I



of the tube flattens and one edge folds over the other, thus covering many of the zooids at the edge of the tube.

b. *The hydroid colony*

The colony consists of two, or perhaps three kinds of individuals: gastrozooids, gonozooids and possibly dactylozooids. The gastrozooids are found only at the rim of the worm tube. In some colonies the gastrozooids are evenly distributed around the aperture of the tube, while in others they are clumped on the side of the tube which will fold under when the worm retires. The gonozooids are located on the body of the tube, apparently never on the rim. They are attached to the gastrozooids and to each other by an anastomosing system of naked hydrorhizae,

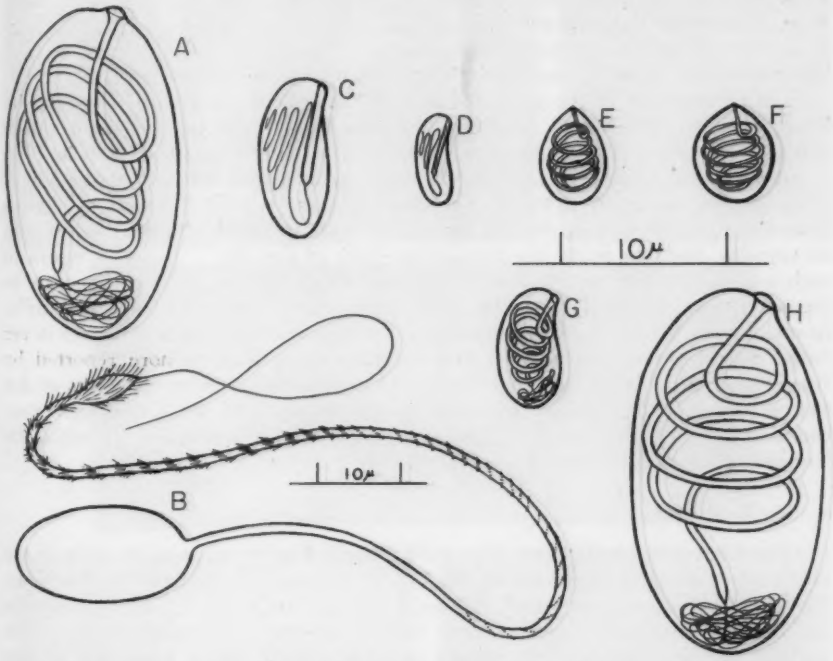


TEXT FIGURE 2. Diagrammatic longitudinal section through a gastrozooid of *Proboscidactyla* sp.

whereas the hydrorhizae of typical Anthomedusae always have a perisarc. The gonozooids, at the seasons we observed them, usually bore from one to four medusa buds in various stages of development. The whole colony may extend from the rim of the tube to a distance of 1.5 cm. down its length. At the edges of the colony farthest from the gastrozooids were found very short, capitate zooids which may have been dactylozooids, although their nematocysts are identical to the gonozoooids, as is their appearance, except that they are smaller and lack medusa buds. Text Figure 1 represents a colony in place upon a sabellid tube. Several of the worm's tentacles are shown as cut away to allow a better view of the colony, and the number of tentacles represented on the worm is considerably reduced from the actual condition.

c. *The gastrozooids*

The gastrozooids are about 0.8 mm. tall, usually less than 0.1 mm. in diameter, and possess two tentacles located at about ¾ the distance from base to top. These tentacles arise from a common area but separate immediately upon becoming distinct from the body. They are highly extensible and may stretch to about 2 mm. in length. The tentacles are solid and their endoderm is in contact with the endoderm of the body. Text Figure 2 is a diagram of a longitudinal section through a gastrozooid showing the relationship of the endoderm of the tentacles and



TEXT FIGURE 3. Nematocysts of *Proboscidactyla* sp.: a–e, of the gastrozooid; f–h, of the medusa. (a. unexploded macrobasic eurytele; b. exploded macrobasic eurytele; c. large microbasic eurytele; d. small microbasic eurytele; e. desmoneme; f. desmoneme; g. small macrobasic eurytele; h. large macrobasic eurytele.)

body. The "head" is separated from the "body" by a distinct "neck" and is capped by an oval battery of nematocysts. The "head" and "neck" are curved, making an arc of 90°, and the mouth is terminal, facing the side of the "body" on which the tentacles are located. The "body" is spindle-shaped, being largest at its midpoint and tapering at its lower end, decreasing to the size of the hydrorhizae, or about 0.03 mm. Plate 2, Figures 3 and 4, are photographs of living isolated gastrozooids. The gastrozooids are oriented around the top of the tube so that

they always face the tube opening. Plate 1, Figure 2, shows a living colony on a tube from which the worm has been removed.

d. *Nematocysts of the gastrozooid*

The types and approximate sizes of the nematocysts of the gastrozooids are given below. The measurements reported for these nematocysts and for the medusa are for discharged capsules. The gastrozooids possess (see Text Fig. 3a–e):

1. Desmonemes: $8 \times 4\ \mu$.
2. Telotrichous macrobasic euryteles: $22 \times 11\ \mu$.
3. Large microbasic euryteles: $11 \times 4\ \mu$.
4. Small microbasic euryteles: $6 \times 2\ \mu$.

The macrobasic euryteles occur only in the nematocyst pad on the head.

The nematocysts of the gastrozooid of *Willia stellata* have been described by Russell (1938), who found nematocysts similar in size and type to those above, with the exception of the macrobasic euryteles. Russell did not find these, but found instead macrobasic mastigophores which were armed throughout the length of the hampe. In regard to the mastigophores he says (p. 145), "In some specimens treated with acetic acid or distilled water the distal end of the hampe swelled into an ampulla, like that in the macrobasic euryteles of Zanclea." Russell's figure of such a capsule seems to illustrate a eurytele which appears identical in type to the macrobasic euryteles (Text Fig. 3a, b) found in the Californian Proboscidactyla. In making our preparations of nematocysts we have used sea water and have never found mastigophores; we suggest that the macrobasic mastigophore reported by Russell is really a macrobasic eurytele. This would make the nematocysts of the gastrozooid of *Willia stellata* identical in type to those of the Californian Proboscidactyla. Further, macrobasic mastigophores are not known for gymnoblastic hydroids other than as reported by Russell above.

e. *The gonozooids*

Mouthless gonozooids (Text Fig. 4 and Plate 2, Fig. 5) occur on the sides of the worm tubes, attached to one another and to the gastrozooids by naked hydrorhizae. They may occasionally be 1 mm. tall but frequently are shorter. The gonozooids are capitate, the distal sphere being heavily studded with nematocysts. The medusae are borne at about the middle of the gonozooid, and a maximum of four medusa buds may be present. These buds apparently arise successively, so that

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PLATE II

FIGURE 3. Isolated living gastrozooids of *Proboscidactyla* sp. removed from the colony shown in Plate I, Figure 2 (ca. 50 ×).

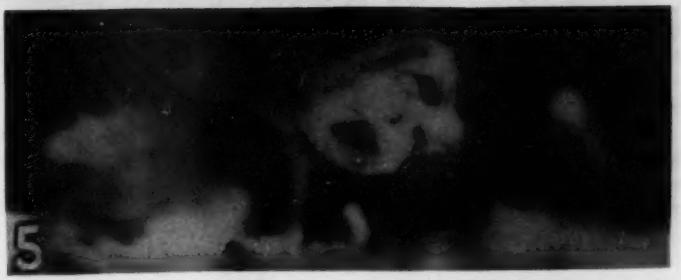
FIGURE 4. A single living gastrozooid of *Proboscidactyla* sp. removed from the colony shown in Plate I, Figure 2 (ca. 50 ×).

FIGURE 5. A living gonozooid of *Proboscidactyla* sp. bearing several medusa buds (ca. 35 ×).

FIGURE 6. A living pentamerous medusa of *Proboscidactyla* sp., oral view, one day after release (ca. 50 ×).

FIGURE 7. A living tetramerous medusa of *Proboscidactyla* sp., one day after release (ca. 50 ×).

PLATE II

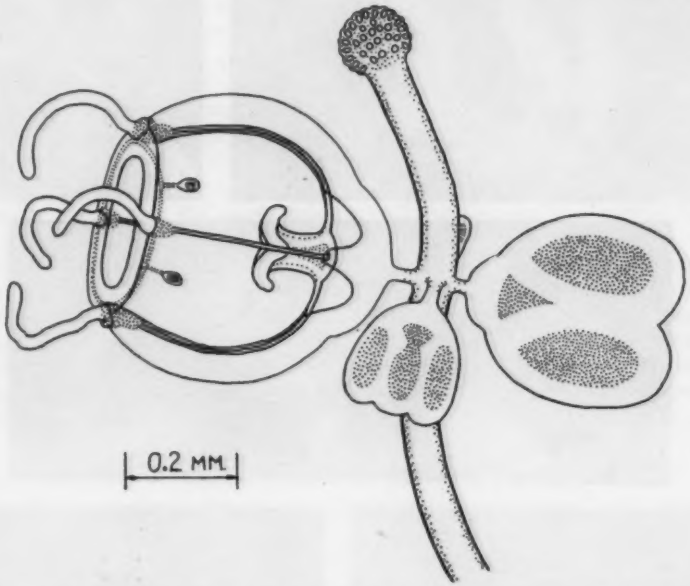


if four are present there are always a large one and three successively smaller ones arranged as in Text Fig. 3. In our specimens the larger medusa buds were seen to pulsate vigorously for a day or two before they were released. Many of the gonozooids possessed 1, 2 or 3 immature buds with no pulsating medusae.

The nematocysts of the gonozooids are the same as those of the gastrozooids except that no small microbasic euryteles are present.

f. *The medusae*

In the course of this study we have seen 25 released medusae. With a single exception, each medusa possessed four primary radial canals, the exception having



TEXT FIGURE 4. A gonozooid of *Proboscidactyla* sp. bearing medusa buds in different stages of development.

five. Plate 2, Figure 6, is a photograph of the living, one day old, pentamerous medusa, and Plate 2, Figure 7 illustrates one of the tetramerous specimens. The medusa possessing five canals was noted before its release and occurred on a gonozooid which also bore normal tetramerous buds. Text Figure 5 shows a medusa as it appears shortly after release, at which time the medusae are about 1.3 mm. tall and 1.0 mm. in diameter. There are four interradial cnidothylacies (nematocyst sacs) which contain large and small macrobasic euryteles, and four tentacles possessing desmonemes and small macrobasic euryteles. The medusa with five radial canals had five tentacles. In no case in the immature medusae which we observed did we find any sign of branching of the radial canals. The ring

canal is represented by a solid cord, to which each of the interradial nematocyst sacs is attached by a strand of endodermal cells. The tentacles are hollow, inserted into the bell by means of large orange-brown pigmented endodermal bulbs, and are marked by nearly regular circular ridges which contain nematocysts. The stomach is four-lobed, each lobe leading into a radial canal. The manubrium is also four-lobed and hangs free under the bell. No statocysts or ocelli have been observed.

All medusae examined were very immature, and neither sections nor microscopic examination of the whole medusa showed any signs of the location of the gonads.

g. *Nematocysts of the medusa* (Text Fig. 3f–h)

1. Desmonemes: $6 \times 5\ \mu$.
2. Large telotrichous macrobasic euryteles: $25 \times 11\ \mu$.
3. Small telotrichous macrobasic euryteles: $8 \times 4\ \mu$.

No microbasic euryteles were found in the medusae.

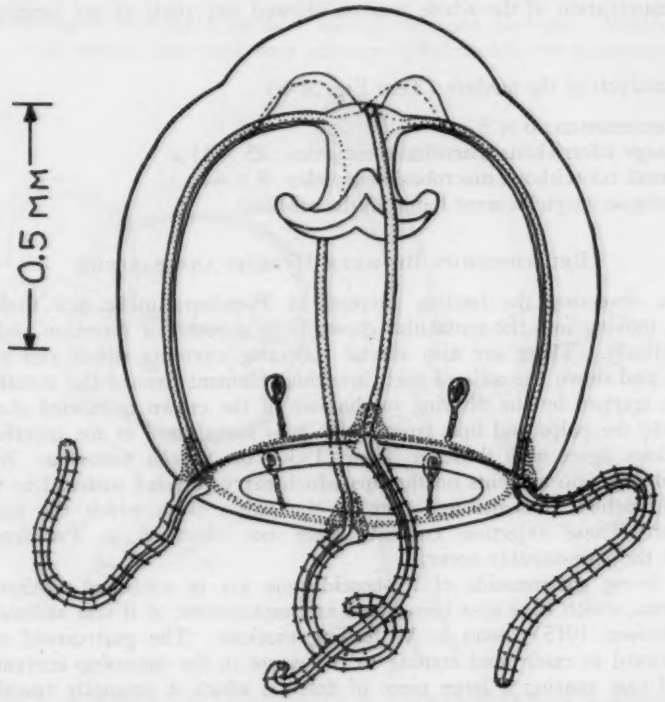
Relationships Between Hydroid and Sabellid

Upon observing the feeding currents of Pseudopotamilla, one finds strong currents moving into the tentacular crown from a posterior direction and passing out anteriorly. There are also special collecting currents which run along the pinnules and down the axis of each branchial filament toward the mouth. Food which is trapped by the filtering mechanism of the crown is carried along these paths onto the palps and lips, from which it is transferred to the mouth. These observations agree with those of Nicol (1930) on *Sabella pavonina*. Nicol also described rejection currents on the lips which carry rejected material to the bases of the branchial filaments and then to the palps, from which the material is discarded. These rejection currents were not observed in Pseudopotamilla, although they presumably occur.

The living gastrozooids of Proboscidactyla are in continual motion. These movements, which have also been noted as characteristic of *Willia stellata* (Gosse, 1859; Brown, 1915), seem to be feeding motions. The gastrozooid may lean far backward to catch food coming to the worm in the incoming current, in one observed case snaring a large piece of detritus which it promptly transferred to its mouth. The gastrozooids also lean forward, sometimes embracing the bases of the branchial filaments with their tentacles; in other cases they lean far into the aperture of the tube and quickly move their tentacles over the surfaces of the lips and palps of the worm. In the cases where the gastrozooids embraced the branchial filaments they were observed actually to remove particles of food, which the worm had collected, from the groove in the axis, which food they promptly devoured. In other cases they searched the surfaces of the lips and palps, and seemed to be gathering food from the currents there. The gastrozooids swing their bodies and tentacles in movements which apparently cover every available area in search of food particles. The rejection currents also presumably carry food to Proboscidactyla, which is in an ideal position to reach into these currents; certainly the area where the rejection currents occur are well covered by the gropings of the

tentacles. Thus this hydroid, living as it does on a host which possesses strong ciliary feeding currents, is able to utilize these currents to supply food for itself.

Many individual gastrozooids were observed whose bodies were distended with food particles as in Plate 2, Figure 3. When such individuals were sectioned, the contained material appeared to be eggs in various stages of digestion. Uchida and Okuda (1941) reported that *P. flavicirrata* readily ate the eggs of its host, *Potamilla myriops*, as the worm spawned. We do not know the source of the



TEXT FIGURE 5. A medusa of *Proboscidactyla* sp. shortly after release.

eggs contained by the Californian Proboscidactyla; perhaps they were the eggs of the host or perhaps they were foreign.

The two-tentacled condition of Proboscidactyla seems to fit it well for its way of life as a commensal. These tentacles are unusually maneuverable and active for a hydroid, and their location on the gastrozooid enables it easily to lean between the branchial filaments, whereas a larger number of tentacles might be a handicap by becoming entangled with the worm. Also the great length to which the tentacles can extend allows the hydroid to cover more territory and thus increases the amount of food it can capture. We have never found this hydroid associated with any animal except *Pseudopotamilla ocellata*.

Discussion

The hydroid described in this paper is similar to those of *Proboscidactyla stellata* and *P. flavicirrata*, but differs from them in that the Californian Proboscidactyla apparently never has gonozooids arising from the gastrozooids. Uchida and Okuda (1941, p. 435) report for *P. flavicirrata*, "The blastostyles are sometimes branched off from the gastrozooids," and Brown (1915, p. 167) says of *P. stellata*, that in the colonies he observed one gonozooid arose ". . . from the base of each polyp at the back," although Hincks (1872) figures gastrozooids and gonozooids arising separately in that species. In all other features of their morphology these three hydroids apparently are remarkably similar. We have found no gastrozooids bearing more than two tentacles, although Brown (1915) and Uchida and Okuda (1941) report occasional individuals with three or more tentacles.

The habitats of all threee hydroids also show remarkable similarities, *P. stellata* living on *Potamilla torelli*, *P. flavicirata* on *Potamilla myriops* and the Californian Proboscidactyla on *Pseudopotamilla ocellata*.

As for the immature medusae described by us, there is little or no way to distinguish them with certainty from other species of Proboscidactyla. The young medusae of *P. stellata* are predominantly hexamerous, although an occasional pentamerous one is found, while those of *P. flavicirrata* vary from tetramerous to hexamerous. *P. mutabilis* is also highly variable. In the Californian form we have found mostly tetramerous medusae as mentioned above. There is an important difference, however, between the Californian species and *P. flavicirrata*. Uchida and Okuda (1941) described the presence of minute eye-flecks on the abaxial faces of the tentacle bases in immature *P. flavicirrata*. These eye-flecks become obscure or are lost in the adult. We have found no such eye-flecks in the Californian material, nor have such flecks been reported for any other member of the family. The presence of these eye-flecks in *P. flavicirrata* necessitates a change in the family definition, which we suggest should read as follows:

Proboscidactylidae:

> Limnomedusae without statocysts; stomach with perradial lobes extending along the proximal parts of the radial canals; gonads surrounding the stomach and its lobes; radial canals branched in the adult; interradial cnidothylacies present; tentacular bulbs usually without ocelli except in young stages of certain species.

Just where Mereschkowsky's hydroid, *Monobrachium parasitum*, fits into this group is not known. Unfortunately, the descriptions of the medusa of Monobrachium are very incomplete, and beyond the fact that the radial canals branch little is known of it. If Monobrachium is a true Proboscidactyla, then we would have a rather striking example of divergent evolution among the hydroids of the genus, some of them being specialized for life as commensals with sabellids and others specialized to live with clams. The hydroid of Monobrachium differs from that of Proboscidactyla in lacking the pronounced "neck" and in possessing but one tentacle. Also the medusae in Monobrachium arise directly from the hydrorhizae rather than from gonozooids so it is possible that Monobrachium will prove not to be a Proboscidactyla and may not even belong to the Proboscidactylidae. But

it will be interesting, should Monobrachium be shown to be a true Proboscidactyla, to see whether this rather unusual hydroid also has a medusa which is divergent, or whether the medusa is of the normal pattern while the hydroid generation alone has diverged.

The specific identification of our Californian hydroid must await further information. Only one Proboscidactyla, *P. occidentalis*, a medusa, has ever been reported from Californian waters. This was described by Fewkes (1889) from Santa Cruz Island. There is nothing in Fewkes' description which enables us to identify our form with his. This is not unusual when one considers the generalized characters of the immature medusae of this group; indeed, our form could be identified with almost any other Proboscidactyla as readily as it could be with Fewkes' species. The closeness of the localities (Monterey Penninsula and Santa Cruz Island) suggests that the Californian Proboscidactyla described by us may be identical with *P. occidentalis*, but until further stages in the development of our form are known this identification must remain speculative.

There still remain species of Proboscidactyla for which no hydroid stages are known, among which one American form merits attention. This is *Proboscidactyla* (= *Willia*) *ornata* (McCrady), a medusa which has been reported from the Woods Hole region. Hargitt (1904, p. 40) reports it as, "More or less common at irregular intervals. Occasionally taken in numbers in the Eel Pond and in the tow of the harbor." There are also at least two sabellids (*Parasabella microphthalma* (Verrill) and *Pseudopotamilla oculifera* (Leidy)) in the Woods Hole area which would seem to be possible hosts for the "Lar" stage of *P. ornata*. The authors hope that the attention of biologists at Woods Hole may be attracted to this problem, and that the hydroid of *P. ornata* will not long remain unknown.

Summary

1. The history of the Proboscidactylidae is summarized and changes are suggested in the definition of the family.

2. A new Californian hydroid is described as *Proboscidactyla* sp. This hydroid may be that of *Proboscidactyla occidentalis* Fewkes.

3. The new hydroid lives as a commensal upon the tubes of a sabellid polychaete, *Pseudopotamilla ocellata* Moore.

4. The commensal relations of the hydroid to the host worm are described, especially the utilization by the hydroid of the feeding currents produced by the worm.

5. The relationship of Proboscidactyla to *Monobrachium parasitum* is discussed.

Acknowledgments

The authors wish to express their gratitude to Dr. C. M. Yonge, University of Glasgow and Visiting Professor, University of California, 1949, Mr. F. S. Russell, Director of the Plymouth Laboratory, and Dr. R. I. Smith, University of California, for their suggestions and advice, and to the director and staff of the Hopkins Marine Station for considerations shown. We also wish to thank Mr. D. P. Abbott, University of California, for the photographs of the living medusae, and Dr. Olga Hartman, Hancock Foundation, University of Southern California, for the identification of the sabellid.

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THE DEVELOPMENT OF RECIPROCAL ANDROGENETIC FROG HYBRIDS [1]

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The role of the cytoplasm in development has long been of interest to embryologists. As early as 1898, Driesch described echinoderm hybrids as purely maternal and concluded that early development is determined by the egg cytoplasm. Conklin (1908) wrote that early development, which includes polarity, symmetry, type of cleavage, relative positions and proportions of future organs, is predetermined in the cytoplasm of the egg. Conklin pointed out that these differentiations arise during ovarian history as a result of the inter-action of the nucleus and the cytoplasm, and thus both play a part in the predetermination of the cytoplasm. These conclusions were based by Conklin on his study of Ascidian eggs (1905) and Wilson's studies of Dentalium and Patella (1904).

One approach to the question of the role of the cytoplasm in development has been the study of amphibian embryos obtained by fertilizing, with foreign sperm, eggs whose maternal chromosomes have been inactivated or removed. P. Hertwig (1916) was one of the earliest workers in this field but it was Baltzer (1920) who initiated the great bulk of this work, using androgenetic Triturus hybrids. These developmental studies have demonstrated the major role of the nucleus in development but the part played by the cytoplasm has not been clarified. I believe that one reason for the failure to demonstrate the cytoplasmic role may be due to the fact that a comparison of reciprocal androgenetic hybrids and androgenetic controls has not been made. Differences in development which might appear in such a comparison may well be ascribed to the action of the cytoplasm. Suitable for such a study are the two morphologically and physiologically distinct species, *Rana pipiens* Schreber and *Rana palustris* Le Conte. Moore (1941) has shown that reciprocal hybrids of these species develop normally and metamorphose, with the hybrids intermediate in appearance between the two species.

The development of the reciprocal androgenetic hybrids between these two frog species will be described in this paper. Their development will be compared with each other and with their androgenetic controls, with the hope that some information may be obtained on the role of the cytoplasm in early development.

I wish to thank Professor L. G. Barth for his interest and guidance in this work.

Materials and Methods

Eggs and sperm of *R. pipiens* and *R. palustris* were used in these experiments. The eggs were obtained by either pituitary induced ovulation or from females col-

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lected in the breeding season with the eggs in the oviducts. Sperm were obtained by cutting up the testes in a small quantity of spring water. Approximately 20 minutes after fertilization the eggs were examined to see if the region where the second polar body comes off was visible. This region, which is called the "Keimpunkt" or "Eispek" by the German authors, is a very small, round, dark indentation on the surface of the animal pole and marks the position of the second maturation division spindle (Fig. 1). Using the method of Porter (1939), the maternal chromosomes were removed by inserting the tip of a sturdy glass needle under the Eispek and lifting the second maturation division spindle out in a small exovate. The eggs subsequently cleaved with only the paternal chromosomes. This method of removal of the maternal chromosomes is successful 97 per cent of the time. The removal of a small exovate does not injure the egg nor alter its later development. This was tested by removing a small amount of protoplasm a short distance from the Eispek. No effect on subsequent development was noted. At the early blastula stage the androgenetic embryos, marked by the small exovate, were separated from the diploid embryos. Only blastulae in which the operation

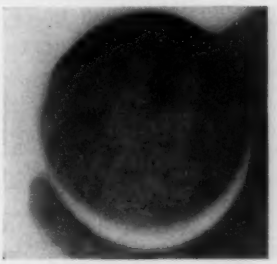


FIGURE 1. The egg of *Rana palustris* 20 minutes after fertilization, showing the small, dark Eispek at the animal pole.

had been well performed were maintained. Approximately equal numbers of diploid blastulae were kept as controls. The embryos were raised in a 12° C. cold room.

The embryos were fixed in Smith's fluid, sectioned at 10 micra, stained with the "Nuclealfarbung" method of Feulgen, and counterstained with light green (Moore and Ryan, 1940). The sectioned embryos were studied and chromosomal counts made. To check on the interpretations, certain embryos were restained with a triple stain recommended by Dr. S. M. Rose. This consisted of picric acid to stain the yolk and endoderm, blau schwartz to stain cell walls and ectoderm, and Orange G to stain both cytoplasm and mesoderm. The stages used to describe the development of the embryos are those of Pollister and Moore (1937).

In referring to individuals, the specific names will be shortened to *pip* and *pal*. The first name will indicate the egg, and the second name the sperm. If the maternal chromosomes were removed, the first name will be in parentheses, indicating a haploid embryo. For example, pip pal means that a pipiens egg was fertilized by a palustris sperm, resulting in a diploid hybrid. In a (pip) pal embryo

the maternal pipiens chromosomes have been removed, resulting in an androgenetic embryo with palustris chromosomes in pipiens cytoplasm.

In a study of androgenetic hybrid embryos, several controls are needed. These controls are the normal diploid embryos, the diploid hybrid embryos and the androgenetic embryos. In order to simplify the description of the experimental material, this material will be divided into two parts. The first part will be a description of those embryos in which the egg of *R. pipiens* was used, and the second part will deal with those embryos in which the egg of *R. palustris* was used.

The Development of (pip) pal Embryos and Their Controls

In this experiment eggs obtained from one Vermont pipiens were used. Some of the eggs were fertilized by sperm from one Vermont pipiens. The remainder of the eggs were fertilized by the combined sperm of two palustris from Woods Hole, Mass. The Eispek was removed from some of the eggs. A total of 204 (pip) pal, 112 (pip) pip, 145 pip pal and 40 pip pip embryos were made and studied. The results from this one large experiment were similar to preliminary experiments when four different pipiens females and five different palustris males were used.

Since the diploid embryos pip pip and the diploid hybrids pip pal were perfectly normal, no description is needed of their development. However, the androgenetic control embryos (pip) pip develop abnormally and a brief description, based on my material, follows. Porter (1939) has described the development of androgenetic pipiens in detail.

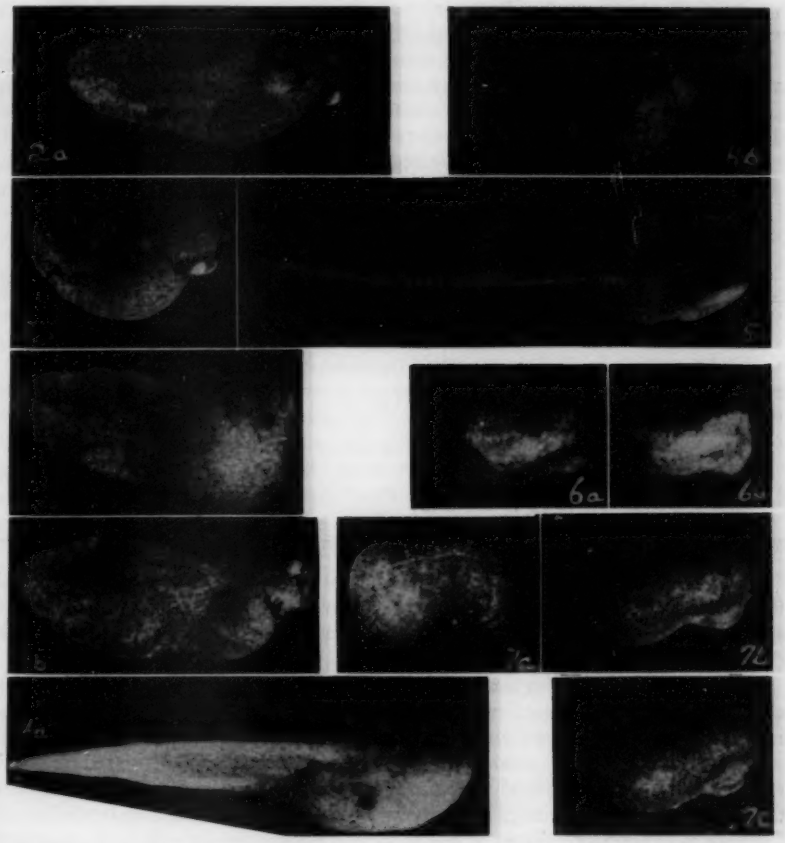
Androgenetic pipiens eggs cleave and gastrulate normally. Their development is delayed during gastrulation. A few embryos begin to cytolyze during the neural fold stage. At the time of closing neural folds many haploids have smaller neural folds than the diploids, and are retarded in development. When the diploid controls pip pip reach stage 19 (heart beat), the haploid (pip) pip are in stage 18 (muscular contraction). When the diploid controls reach stage 20 (gill circulation), the haploids resemble a late stage 18. A few show heart beat and tiny gill projections, but the remainder show neither. All have very large pronephric swellings, wrinkled abdominal epidermis, deep stomadeal pit and a short, broad body (Figs. 2a and 2b). When the diploid controls reach stage 22 (perforated mouths and with the gills almost overgrown by the operculum), the haploids have an open mouth and a small operculum growing over the tiny gill projections. Some of the haploids are very swollen and bloated in appearance, and some are collapsed and wrinkled. When the controls are in stage 23 (horny teeth and with the operculum completely covering the gills), most haploids show no further differentiation than described above. They are either swollen or collapsed and appear to be dying (Figs. 3a and 3b).

The percentage of haploid survival is very high up to tailbud stage. After this stage many embryos begin to die, and differentiation is retarded and incomplete, even though survival may continue for some time.

From the foregoing description it is evident that haploid development is distinct from diploid development. Haploids are easily recognized by their characteristic development, which is retarded and abnormal to a varying degree.

The development of the androgenetic hybrid embryos (pip) pal was markedly different from the control haploid pipiens development. The embryos were re-

tarded during gastrulation so that when the control pip pal embryos had reached an early yolk plug stage, these embryos still had a semicircular dorsal lip. When the (pip) pal embryos reached an early yolk plug stage, some of the yolk cells appeared vacuolated. A small normal yolk plug was formed in all, and up to this time no cytolysis had occurred. By the time the control hybrids were in an early neural fold stage, cytolysis of the haploid hybrids began. The (pip) pal formed a flat



FIGURES 2a and 2b. Androgenetic pipiens embryos whose diploid controls are in stage 20.
FIGURES 3a and 3b. Androgenetic pipiens embryos whose diploid controls are in stage 23.
FIGURES 4a and 4b. Androgenetic pipiens embryos showing the degree of variation in differentiation. Embryos a and b are the same age and from the same experiment.
FIGURE 5. The diploid pipiens control embryo for Figures 4a and 4b.
FIGURE 6a. Elongated (pip) pal neural plate embryo.
FIGURE 6b. A (pip) pal embryo showing the exposed gray cells of the neural plate region.
FIGURE 7. Control embryos for the (pip) pal embryos figured in 6a and 6b. a, (pip) pip.. b, pip pip. c, pip pal.

neural plate, and the embryo elongated slightly (Fig. 6a). In those embryos in which the yolk plug was still evident, the yolk plug cells had begun to cytolyze. The wave of cytolysis spread anteriorly, eventually involving the whole neural plate. In embryos in which the yolk plug was a mere slit, a few loose cells appeared on the posterior edge of the neural plate, and again the wave of cytolysis progressed anteriorly. When the vitelline membrane was removed from an embryo, these loose cells came off with it. This revealed the neural plate region as a mass of gray cells, the pigmented ectodermal cells having been sloughed off (Fig. 6b). These (pip) pal embryos can be compared with their controls in Fig. 7.

The (pip) pal embryos were still neural plate embryos when pip pal had closed neural folds. Only 20 (pip) pal survived until the pip pal had reached a tailbud stage. Of these, 3 were still neural plate embryos, 13 showed very slight neural folds and 4 were normal diploid embryos. No further differentiation of (pip) pal embryos occurred, and death followed shortly. This retardation and stoppage of development of (pip) pal embryos is shown in Table I.

TABLE I

A record of the stage of development of (pip)pal embryos and their controls on successive days

Age in days	The stage of development of			
	(pip)pal	(pip)pip	pip pal	pip pip
5th day	11	11–12	12	12
6th day	12	12	12	12–13
7th day	12	12–14	13	13–14
8th day	12–13	13–16	14–15	15–16
9th day	13	14–17	14–16	16–17
10th day	13–14	16–17	16–17	17

Seven (pip) pal embryos were sectioned, varying from a neural plate to a faint neural fold stage, the latter representing the maximum development. A description of these embryos follows after a description of the normal picture in a diploid early neural fold embryo of *R. pipiens*.

A normal embryo is diagrammed in Figure 8. The neural ectoderm is greatly thickened in the anterior and middle regions where it will give rise to brain and neural folds. The notochord in the anterior region is not differentiated. In progressively more posterior sections it gradually becomes differentiated from the mesoderm until it is completely separated from all three layers. The mesoderm in the anterior region continues dorsally as chorda-mesoderm, and extends laterally and somewhat ventrally. Toward the posterior region the mesoderm is completely separated from the notochord and extends a short way laterally. The gut anteriorly has a large lumen which is not roofed by endoderm. Its lumen gradually becomes smaller and completely roofed by endoderm in the posterior region. A liver is present. There are very few cells in mitosis, and no pycnosis nor abnormal nuclei are apparent.

The (pip) pal neural plate embryo is strikingly different. The following description of embryo (pip) pal No. 8, Figure 17, is typical. The neural ectoderm

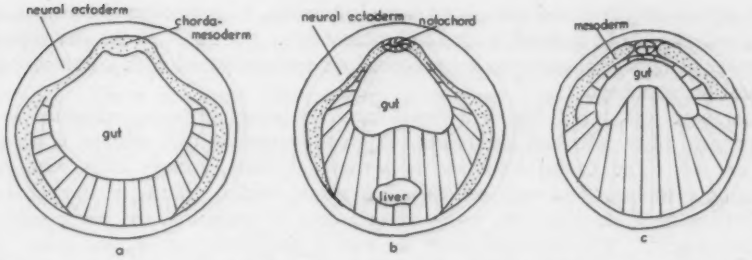


FIGURE 8. Diagram of a diploid pipiens early neural fold embryo, showing an anterior section in a, a middle section in b, and a posterior section in c.

is greatly thickened in the anterior and middle regions of the embryo. Pycnotic nuclei are present in the anterior neural ectoderm which gives rise to brain. In the most anterior region the pycnotic nuclei number about 20 per section. They decrease in number until, in approximately the region where brain and neural tube would join, none are present. In some of these nuclei the chromatin is clumped together. In others the center of the nucleus is faintly stained, and around the periphery is heavily stained chromatin. Still other nuclei are small, condensed and heavily stained.

In the anterior region of the embryo the mesoderm is first distinguishable as a separate layer laterally. It continues dorsally where it fuses with the ectoderm and endoderm. There is no differentiation into chorda-mesoderm. Near the middle of the embryo the mesoderm is separate from the ectoderm and endoderm dorsally and extends laterally and somewhat ventrally. Toward the posterior part of the embryo the mesoderm is a complete layer ventrally as well as dorsally. No notochord is present here or anywhere thoroughout the whole embryo.

The gut along its whole length has a very small lumen which is roofed with endoderm. No liver is present. Both the extent of the mesoderm and the roofing of the gut are characteristic of older diploid closed neural fold embryos, not of neural plate embryos. In contrast to a normal diploid neural plate embryo, many

TABLE II

A comparison of the developmental features of each sectioned (pip)pal embryo with a normal diploid neural plate embryo. The lengths of various organs are measured in micra from the anterior end of each embryo

Embryo	Total length	Gut extends from	Total length of gut	Notochord extends from	Liver extends from
pip pip	1730	130–1510	1390	640–1420	350–650
(pip)pal No. 1	1770	380–1320	950	0	0
(pip)pal No. 2	1890	140–1510	1380	0	0
(pip)pal No. 3	1840	220–1640	1430	0	0
(pip)pal No. 4	1740	180–1470	1300	0	0
(pip)pal No. 6	1700	190–1360	1180	0	0
(pip)pal No. 7	1840	250–1720	1480	0	0
(pip)pal No. 8	1710	80–1500	1430	0	0

cells of the ectoderm and mesoderm were in mitosis. Chromosomal counts showed all 7 embryos to be haploid, with 13 chromosomes. In Table II the developmental features of (pip) pal embryos are summarized and compared with a normal pipiens neural plate embryo.

Embryo (pip) pal No. 6 differed from the other embryos sectioned in that the neural plate cells had cytolyzed. A cross section of this embryo is shown in Figure 18. The dorsal ectoderm is a mass of loose, spongy cells with poorly staining cytoplasm and with condensed, pycnotic nuclei. Many of the ectodermal cells have been sloughed off, as no surface coat is present at the outer surface of the loose cells. The whole length of the neural plate shows cytolyzing cells. From one-quarter to one-third of the way from the anterior end of the embryo, all of the dorsal ectodermal layer and possibly some of the underlying mesoderm is involved in this cytolysis. This is difficult to ascertain, as in this region mesoderm and ectoderm are difficult to separate dorsally. More posteriorly, only the ectoderm is involved. Very few cells of this embryo were in mitosis. In Figure 17 the cytoplasm of the neural ectodermal cells is poorly stained and seems altered in character. This change may well presage the sloughing off of these cells as pictured in Figure 18. Embryo (pip) pal No. 1 also showed cytolysis of the dorsal ectodermal cells, but to a minor extent.

From the foregoing description of (pip) pal embryos it is evident that gastrulation is accomplished, and the three germ layers are formed. Neither notochord nor liver differentiate. The embryos remain in an arrested abnormal neural plate stage. Pycnotic nuclei are present in the neural ectoderm which will give rise to brain. Mitotic activity ceases. The ectodermal cells at the dorsal edge of the blastopore round up and begin to slough off. This cytolysis of the ectodermal cells spreads anteriorly until the whole neural plate is involved, and the embryo then dies.

The Development of (pal) pip Embryos and Their Controls

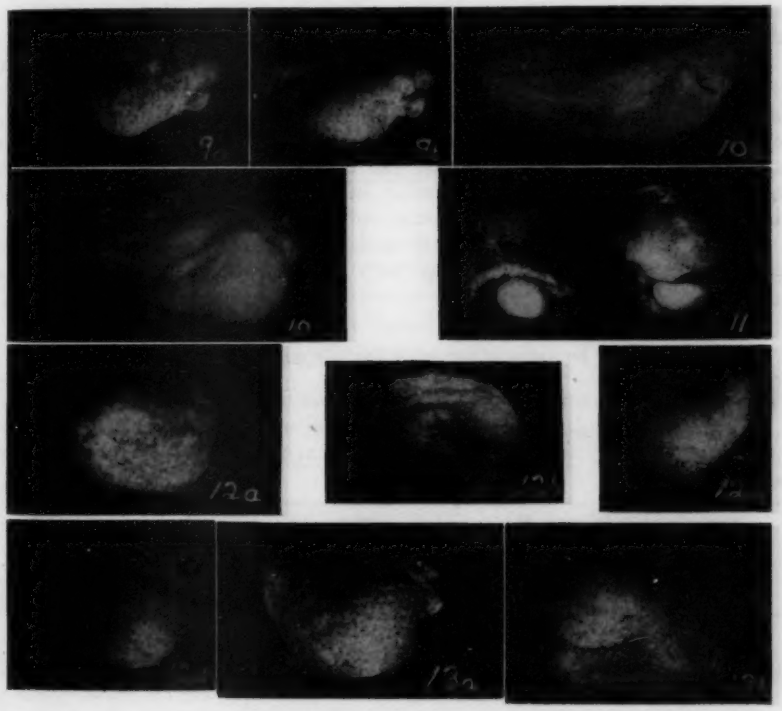
In this cross, 3 experiments were performed at different times. In each experiment eggs were obtained from one palustris female and part of the eggs were fertilized by a palustris male and the remainder by a pipiens male. Thus three different palustris females, 4 palustris males (the combined sperm of two small males were used in one experiment) and 3 pipiens males were used. A total of 304 (pal) pip embryos, 148 (pal) pal, 363 pal pip and 214 pal pal were made and studied.

The diploid embryos pal pal and the diploid hybrids pal pip developed perfectly normally. The (pal) pal embryos were similar in their development to (pip) pip embryos. Figures 9 and 10 show haploid palustris tadpoles. They have short, broad tails, large swollen bellies, and microcephalic heads with optic vesicles, olfactory pits and suckers. The (pal) pal embryos differ from (pip) pip in that their survival to later stages (Table IV) and their degree of differentiation is low. Their maximum development is shown in Figure 10. Only 4 of the 148 embryos survived until the control palustris diploids were in stage 21 (cornea transparent). In these (pal) pal the gills developed as a tiny, short filament, the stomadeum was a very small opening, and the heart was observed beating.

The androgenetic hybrid embryos (pal) pip develop normally up to gastrula-

tion. From the beginning of gastrulation their development is retarded, abnormal and highly variable. The dorsal lip may remain as a crescent and a small neural plate may appear and two small tailbuds may project above the large yolk plug (Fig. 11). These embryos differentiate no further, though they may survive until their controls (pal) pal are in stage 18 and pal pip and pal pal in stage 19. In some embryos a large yolk mass and short anterior projection are present (Fig. 12a).

A few embryos formed dorsal and lateral lips of the blastopore, although no ventral lip was present. In these embryos invagination and overgrowth may occur to such an extent that only a tiny yolk plug is left. Low neural folds may form and close (Fig. 12b) and an anterior projection may develop upon which no morphological head structures are discernible (Figs. 12c and 12d). The percentage of



FIGURES 9a, 9b, 10a and 10b. Androgenetic palustris embryos.
FIGURE 11. Two (pal) pip No. 149 embryos showing abnormal gastrulation.
FIGURES 12a and 12b. Two (pal) pip No. 184 embryos, b showing neural folds.
FIGURES 12c and 12d. Two (pal) pip No. 273 embryos, showing anterior projection and short tail.
FIGURES 13a and 13b. Two (pal) pip No. 184 embryos, showing maximum differentiation reached by any (pal) pip embryo.

TABLE III

A record of the percentage of (pal)pip to reach various degrees of differentiation in the different experiments

	Original number	Percentage of (pal)pip showing				
		Incomplete gastrulation	Complete gastrulation	Neural plate	Neural folds	Anterior projection
Exp. 4/24	34	97.1	0.0	—	8.8	0.0
Exp. 4/19	149	98.6	0.0	57.2	18.8	4.5
Exp. 5/2	121	88.2	10.8	66.7	47.7	12.6
Total	304	92.6	4.1	61.4	29.1	7.0

(pal) pip embryos to reach these various degrees of differentiation are shown in Table III.

A few embryos gastrulated completely, but no differentiation of head structures on the anterior projection occurred. Only two (pal) pip out of all 304 embryos showed greater differentiation (Figs. 13a and 13b). Optic vesicles and suckers are recognizable in one. The percentage of survival of (pal) pip embryos is lower than that of (pal) pal and no (pal) pip embryo survived longer than control stage 19 (Table IV). The retardation of development of these androgenetic hybrid embryos is marked and is summarized in Table V.

Fifteen (pal) pip embryos were sectioned. Thirteen of these ranged in development from an early neural plate stage with a large yolk mass to a closed neural

TABLE IV

A comparison of the percentage of survival of (pal)pip embryos and their controls at various stages of development

	Original number	Percentage of survival when the control pal pip were in		
		Stage 13 6th day	Stage 17 9th day	Stage 18–19 12th day
(pal)pip	304	90.6	31.2	11.1
(pal)pal	148	92.1	40.9	20.4
pal pal	214	100.0	99.6	93.6
pal pip	363	98.2	95.0	93.4

TABLE V

A comparison of the stages of development of pal pip and (pal)pip embryos

pal pip	9	10	11	12	14–15	16	17	18–19
(pal)pip	9	10	10	11	11 with neural plate	11 with neural folds	11 with closed neural folds	11 with anterior projection or closed blastopore with projection

fold stage with an anterior projection. Two were the embryos which have been described as the most advanced. The presence or absence of various organs and their lengths are recorded in Table VI, where they are also compared with data from normal pip pip embryos. In addition, each embryo has been graphically reconstructed in Figures 14, 15, and 16. Each embryo is identified by a number. Those with the same initial numbers, for example 250–1 and 250–2, are from the same experiment and fixed at the same time, so that they are of the same age,

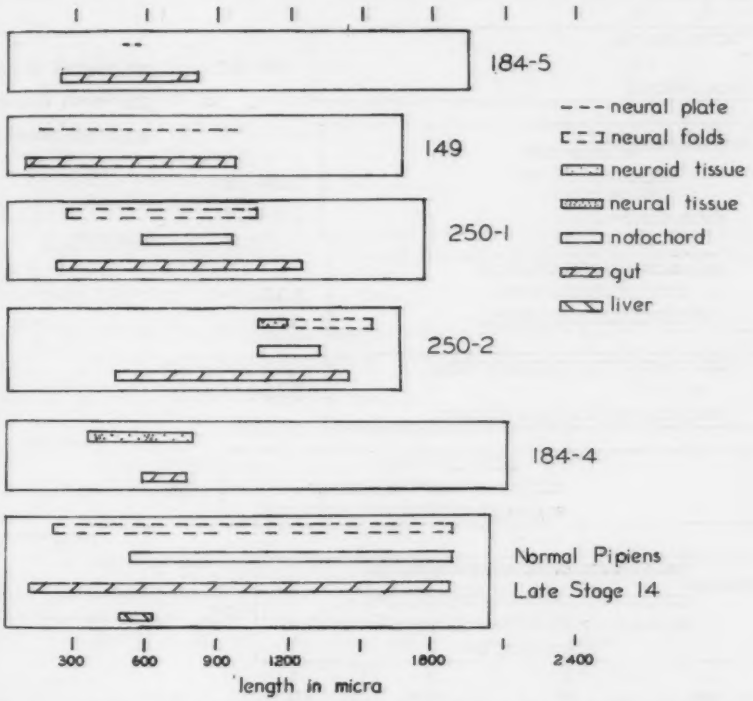


FIGURE 14. Graphic reconstructions of 5 (pal) pip embryos and of a normal late stage 14 pip pip embryo No. 168.

though of various degrees of differentiation. Some of the sectioned embryos with the initial numbers 149 have been shown as whole embryos in Figure 11, 273 in Figures 12c, 12d and 184 in Figures 12a, b and 13a, b.

The five least differentiated (pal) pip embryos are graphically reconstructed in Figure 14 and compared with a normal pip pip late stage 14 (neural folds) whose stage of development they most closely resemble. Their control pal pip embryos ranged in age from stage 14 for embryo 149 to stage 16 (closed neural folds) for embryos 250–1 and 250–2, to stage 19 (heart beat) for embryos 184–5 and 184–4.

A large yolk mass protruded dorsally in the posterior half of each embryo except 250–2.

The five (pal) pip embryos which survived until the control pal pip were in either stage 18 (muscular contraction) or stage 19 are graphically reconstructed in Figure 15, and compared with a normal pip pip early stage 16 embryo whose stage of development they most closely resemble. Each embryo had closed neural folds but a large yolk mass protruded dorsally in embryos 184–8C, 184–7B and 238.

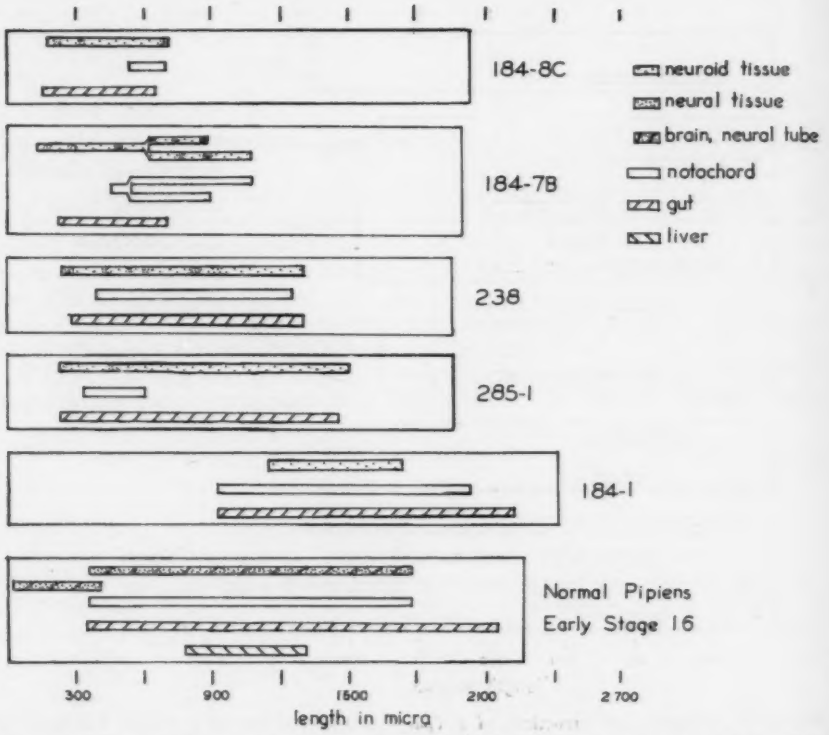


FIGURE 15. Graphic reconstructions of 5 (pal) pip embryos and of a normal early stage 16 pip pip embryo No. 166.

The remaining five (pal) pip embryos also survived until the control pal pip embryos were in either stage 18 or stage 19, but showed greater differentiation. These embryos are graphically reconstructed in Figure 16 and compared with a normal pip pip stage 18 embryo, whose stage of development they most closely resemble. Anterior projections were present in embryos 184–2, 273 and 285–2. Embryos 184–6a and 184–9 were the most advanced (pal) pip embryos shown in Figures 13a and b.

A general description of the internal differentiation of (pal) pip, based on these fifteen sectioned embryos, will now follow.

A neural plate with lateral ectodermal thickenings may differentiate, even though a large yolk plug may be present due to incomplete gastrulation (Fig. 14, embryos 184–5, 149). Neural folds may develop (Fig. 14, embryos 250–1, 250–2) but are very low, with the cells poorly oriented (Fig. 19), contrasting markedly with a normal closing neural fold embryo (Fig. 20). Where these neural folds close, differentiation into neuroid tissue may occur (Fig. 14, embryo 250–2; Fig. 15, embryo 184–1). The term "neuroid tissue" is used to describe a mass of darker

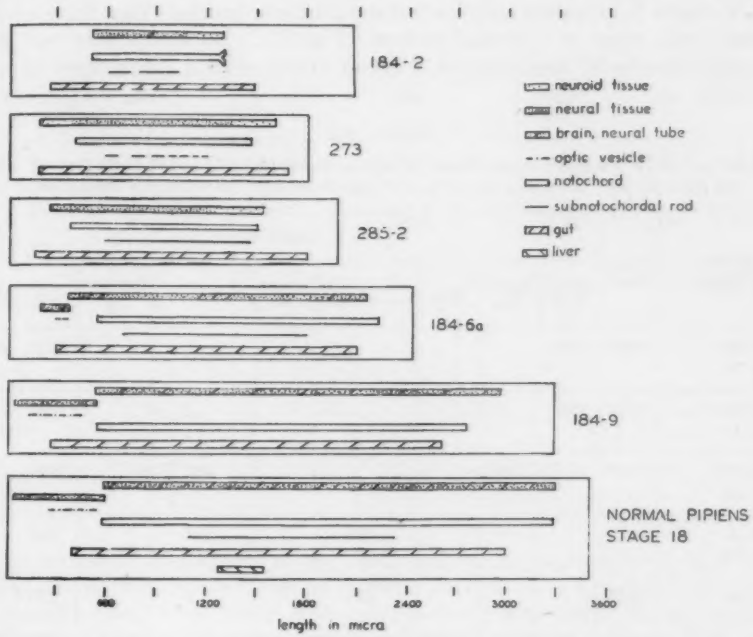


FIGURE 16. Graphic reconstructions of 5 (pal) pip embryos and of a normal stage 18 pip pip embryo No. 613.

staining, poorly differentiated ectodermal cells with scattered pigment throughout. Such neuroid tissue is shown in Figure 21 and can be compared with a normal neural tube in Figure 22.

In some embryos not only is neuroid tissue present, but also neural tissue. The term "neural tissue" is used to define a group of ectodermal cells which are more or less delimited from the surrounding cells by a membrane, and may or may not show a neurocoel (Fig. 23). It is markedly different from a normal neural tube (Fig. 22). Both neuroid and neural tissue are present in varying amounts in the embryos (Figs. 14–16 and Table VI).

In those embryos in which an anterior projection is formed there is a small amount of neuroid tissue and a large amount of neural tissue (Fig. 16, embryos 184–2, 273 and 285–2). The neural tissue may, at times, closely approximate a neural tube (Fig. 26) and in one such embryo (184–2) a neural tube was present for a short distance. This neural tube is thick and abnormal in appearance (Fig. 27) when it is compared with a normal pipiens neural tube (Fig. 28).

Neural tissue, neural tube, brain and optic vesicles are present in the two most advanced embryos (Fig. 16, embryos 184–6a and 184–9). The brain and optic vesicles are very thick walled and abnormal in appearance (Fig. 29) as can be seen by a comparison with a normal embryo (Fig. 30). One of these embryos (184–9) had a thick walled hind brain and paired otic vesicles (Fig. 31) markedly different from those in a normal embryo (Fig. 32). Its neural tube was very abnormal posteriorly, appearing as a round vesicle with a single layer of cells (Fig. 33).

TABLE VI

A comparison of the lengths of organs found in each sectioned (pal)pip embryo with those of normal pip pip embryos. Length is in micra. c = control pal pip. st = stage of development

(pal)pip embryo number	Total length	Length of								
		Neural plate	Neural folds	Brain	Neuroid and neural tissue	Neural tube	Notochord	Subnoto-chordal rod	Gut	Liver
184–5 c-st 19	1960	80					0		570	0
149 c-st 14	1670	830					0		890	0
250–1 c-st 16	1770		790				400		1010	0
250–2 c-st 16	1670				480		240		980	0
184–4 c-st 19	2120				400		0		170	0
184–8c c-st 19	2050				530		170		500	0
184–7b c-st 19	2010				760 / 470 \ 940		440		480	0
238 c-st 19	1970				1070		860		1030	0
285–1 c-18	1970				1290		270		1240	0

TABLE VI.—*Continued*

(pal)pip embryo number	Total length	Length of								
		Neural plate	Neural folds	Brain	Neuroid and neural tissue	Neural tube	Notochord	Subnoto-chordal rod	Gut	Liver
184–1 c-st 19	2440				580		1130		1310	0
184–2 c-st 19	2080				780		800 770 800	350	1230	0
273 c-st 18	1810				1430		1070	510	1490	0
285–2 c-st 18	1970				1290		1120	880	1640	0
184–6a c-st 19	2440			170	1780		1690	1120	1820	0
184–9 c-st 19	3290			500		2430	2230	0	2350	0
pip pip 168 st 14	2050		1700				1380		1680	140
pip pip 166 st 16	2250			410		1510	1510		1870	290
pip pip 613 st 18	3540			570		2730	2730	1240	2610	270

A notochord was absent in the (pal) pip neural plate embryos and in one closed neural fold embryo (Fig. 14, embryo 184–4). The notochord was present in the remaining closed neural fold embryos but it was highly variable in length and bore no relationship to the length of the neuroid and neural tissue (Figs. 14 and 15). In the most highly defferentiated (pal) pip embryos (Fig. 16) a well developed notochord and subnotochordal rod were present (Fig. 26).

A gut with a small lumen was present in all embryos. No liver was present in any (pal) pip embryos. A mesodermal layer was absent in the most poorly differentiated embryo 184–5, but present in all the rest of the embryos of Figs. 14 and 15 and embryo 285–2 of Fig. 16. The remaining embryos of Fig. 16 showed somites. A short pronephric duct was present in the most advanced embryo (184–9). A heart was not present in any (pal) pip embryo.

Chromosomal counts showed the following embryos to be haploid: 184–5, 250–2 and 184–4 of Figure 14; 184–7B and 285–1 of Figure 15; 273 and 184–6a of Figure 16. The remaining 8 (pal) pip embryos were either mitotically inactive or with nuclei so faintly stained that chromosomal counts could not be made. They

are believed to be haploid as their differentiation was similar to other (pal) pip embryos in which chromosomal counts could be made.

Pycnotic nuclei and loose cells were noted in some of the least differentiated and in some of the most highly differentiated embryos. Figure 24 shows such loose ectodermal and mesodermal cells which were scattered in a large fluid filled space about the notochord of embryo 184–1, Figure 15. The pycnotic nuclei (Fig. 25) of these loose cells show either peripheral clumping of the chromatin or they are small, condensed and darkly staining. Pycnotic nuclei were present in ectodermal, mesodermal and endodermal cells (embryo 184–6a, Fig. 16).

Oedema occurred in some of the less differentiated as well as in some of the most highly differentiated embryos. The oedematous spaces were within the ectoderm, between the ectoderm and endoderm, or between the ectoderm and mesoderm. One such large space is shown in Figure 33.

From the foregoing description it is evident that even though gastrulation in (pal) pip embryos may be incomplete, a neural plate with lateral ectodermal thickenings is formed. A shallow neural groove and low neural folds with poorly oriented ectodermal cells is then formed. When the folds close, neuroid and neural tissue are formed. With more complete gastrulation, differentiation into a recognizable neural tube and even into brain may occur. A notochord develops. A gut but no liver is present. A mesodermal layer and even somites are present. Oedema may occur. Pycnosis may be associated with the breakdown of tissues.

Discussion

The discussion will be devoted to the consideration of haploidy in amphibians and of the results obtained by combining a haploid set of chromosomes of one species with the cytoplasm of a different species.

The abnormal development of haploid parthenogenetic and androgenetic amphibian embryos has been ascribed to the condition of haploidy *per se* or to the presence of unmasked lethal or semi-lethal genes. It would seem logical that both factors play a role. A characteristic haploid syndrome may be present. Haploid tadpoles are usually small, with short bodies, broad tails, abnormal eyes, a poorly looped gut and oedema. The degree of differentiation is, however, highly variable. In a large group of (pip) pip embryos the degree of abnormality may vary from extreme to slight. Two such embryos are shown in Figures 4a and

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Plate I

Figure 17. Section of embryo (pip) pal No. 8, 610 micra from anterior end, showing thickened neural ectoderm.

Figure 18. Section of embryo (pip) pal No. 6, 920 micra from anterior end, showing cytolyzing neural plate cells.

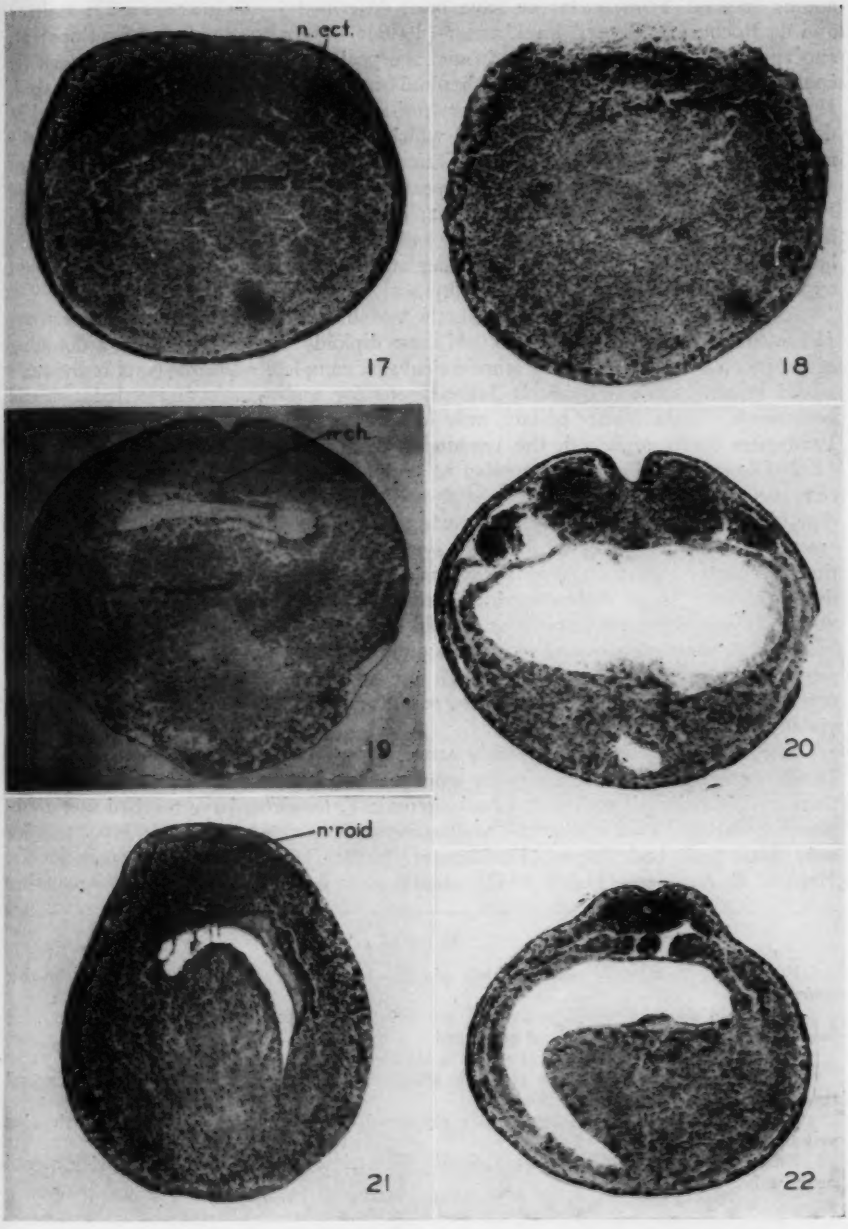
Figure 19. Section of embryo (pal) pip 250–1, 750 micra from anterior end, showing neural folds.

Figure 20. Section of embryo pip pip 168, 530 micra from anterior end showing normal neural folds.

Figure 21. Section of embryo (pal) pip 250–2, 1100 micra from anterior end, showing neuroid tissue.

Figure 22. Section of embryo pip pip 166, 830 micra from anterior end, showing normal neural tube.

PLATE 1



b, which can be compared with their normal control in Figure 5. Such variations among haploid Triturus larvae have been described by Baltzer (1922), Baltzer and de Roche (1936) and Fankhauser (1930). These *variations* of development, superimposed on the haploid syndrome, are probably due to the different genetic constitution of each embryo. In the haploid condition recessive genes are unmasked. If any of these are embryonic lethals or semi-lethals, their effects will be observed in development. In haploid embryos in which the majority of the genes are of the normal type, it is possible that differentiation may go far and that even metamorphosis may be reached. This view is supported by the fact that Baltzer (1922) and Fankhauser (1938) described a metamorphosed haploid *T. taeniatus*. With increasing numbers of semi-lethal and lethal genes, haploid differentiation would become poorer and death occur at earlier stages. Thus genetic differences would explain the variability of haploid development.

As evidence against this hypothesis the viability of diploid parthenogenetic frogs (Parmenter, 1933) has been cited. If these diploids were produced by a doubling of the chromosomes at the first mitotic cleavage, completely homozygous individuals would result. Such individuals, homozygous for semi-lethals and lethals, should be inviable. As a matter of fact, only half of the few parthenogenetic diploids of Parmenter metamorphosed, the remainder dying at an early stage. Kawamura (1939) found that in a large number of diploid parthenogenetic frogs only 17 per cent metamorphosed, and even these showed slight irregularities. The remaining diploids were abnormal and his drawings of some of these diploids markedly resemble embryos with the haploid syndrome. Such results tend to support the hypothesis that unmasked recessive genes play a role in abnormal haploid development. Those embryos which have unmasked lethals and semi-lethals can not survive in either the haploid or diploid condition.

In those parthenogenetic cases where diploidy is restored by the retention of two sets of maternal chromosomes through the suppression of a meiotic division, a certain degree of heterozygosity would result and such diploids might have normal viability.

The injurious effect of haploidy varies from species to species in amphibia. In my experiments, haploid pipiens embryos live longer and differentiate further than haploid palustris embryos. Androgenetic *T. taeniatus* have reached metamorphosis (Baltzer, 1922), whereas androgenetic *T. palmatus* and *alpestris* survive only until limb bud stages (Fankhauser, 1930; Baltzer and de Roche, 1936). Haploid *R. esculenta* (Dalcq, 1932) usually form neurulae, and some develop tiny

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PLATE II

FIGURE 23. Section of embryo (pal) pip 285–1, 430 micra from anterior end, showing neural tissue.

FIGURE 24. Section of embryo (pal) pip 184–1, 1600 micra from anterior end, showing loose cells in fluid filled space about notochord.

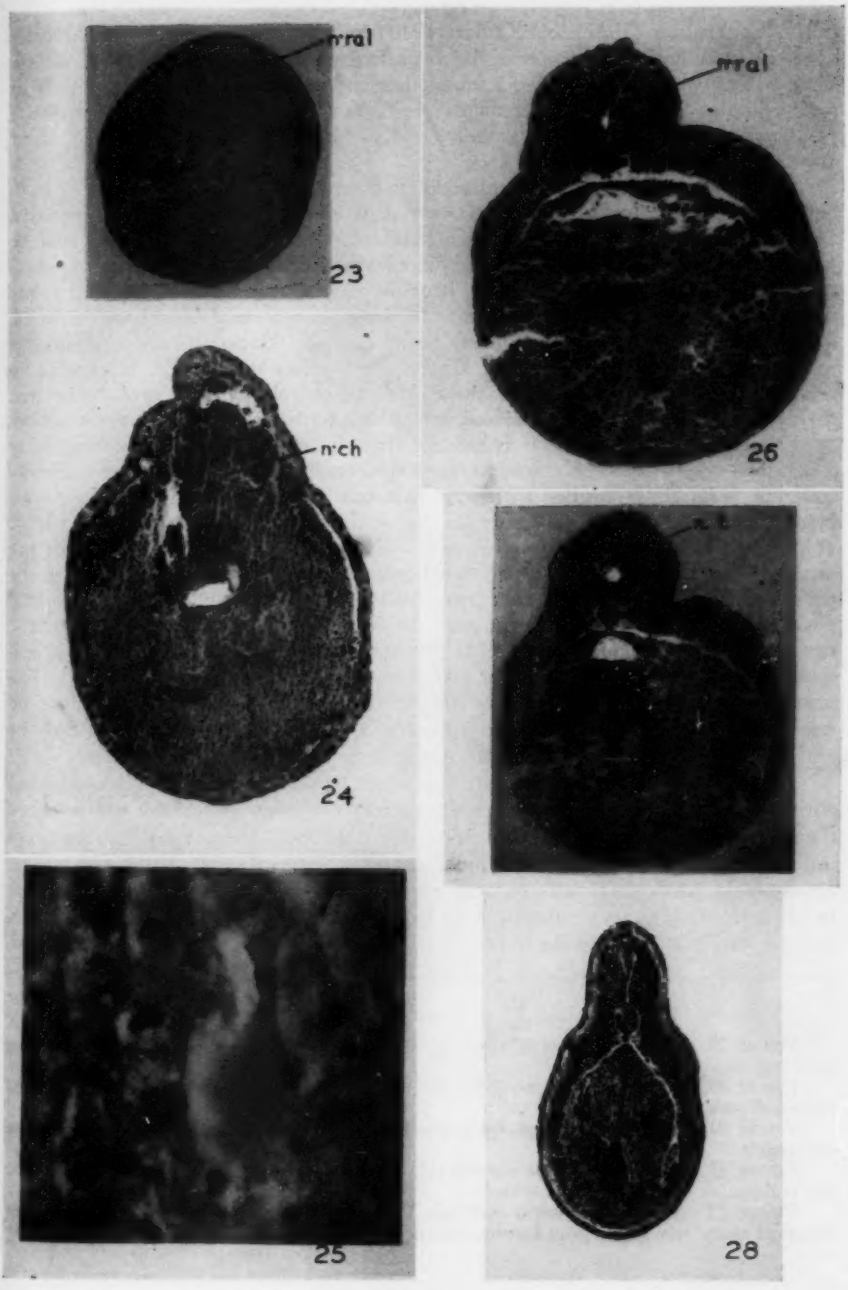
FIGURE 25. Pycnotic nuclei present in the loose cells shown in Figure 24.

FIGURE 26. Section of embryo (pal) pip 184–2, 960 micra from anterior end, showing neural tissue, notochord, and subnotochordal rod.

FIGURE 27. Section of embryo (pal) pip 184–2, 890 micra from anterior end, showing neural tube.

FIGURE 28. Section of embryo pip pip 613, 1270 micra from anterior end, showing normal neural tube.

PLATE II



gills. Fankhauser (1937) and Kaylor (1940) found that in haploid *T. pyrroghaster* approximately half of the embryos die as gastrulae but that others go on to a forelimb bud stage. Stauffer (1945) found that androgenetic axolotl survive only as long as the gastrula and neurula stage. Griffiths (1941) showed that the majority of androgenetic *T. viridescens* embryos die as blastulae and gastrulae, but about 10 per cent form neurulae.

Death during late blastula and early gastrula stages is probably not due to haploidy but to abnormal chromosomal distributions and mitotic aberrations. Because of polyspermy in Triturus, a balanced set of chromosomes may not be present and Fankhauser (1934) has pointed out that such a balanced set appears to be needed for gastrulation and later development. Development to a late blastula or early gastrula stage appears to be under maternal cytoplasmic control, and further differentiation is under genic control. Boveri (1907) noted this in dispermic sea urchin eggs and Moore (1941) has discussed this concept in relation to frog hybrids. All of these findings corroborate the probability that the abnormal development of haploid embryos is partially a result of genic action.

From the data presented it is evident that the results obtained by combining a haploid set of palustris chromosomes with the cytoplasm of *R. pipiens* are markedly different from those results when the palustris chromosomes are in their own cytoplasm. These (pip) pal embryos die at an earlier stage than (pal) pal or (pip) pip. Some process or processes in differentiation are either lacking or are unable to carry out their normal functions. For example, the absence of the differentiation of the neural tube may be explained embryologically by several possibilities. (1). The organizer may be in a weakened condition, so that a neural tube can not be induced. (2). The organizer may be normal but the neural ectoderm may be weakly competent and thus no neural tube is formed. (3). The organizer may be weak *and* the neural ectoderm poorly competent, therefore no neural tube is formed. A definite answer to these three possibilities can only be obtained by xenoplastic implants and transplants of the materials involved.

Similarly, the results obtained by combining a haploid set of pipiens chromosomes with the cytoplasm of *R. palustris* are markedly different from those results when the pipiens chromosomes are in their own cytoplasm. These (pal) pip embryos differentiate much more abnormally than (pip) pip or (pal) pal. The neural ectoderm is particularly affected, as a recognizable brain and neural tube are rarely formed. In these embryos, as in (pip) pal, the competence of the neural ectoderm may be lowered or the inductive capacity of the organizer may be weakened,

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PLATE III

FIGURE 29. Section of embryo (pal) pip 184-9, 210 micra from anterior end, showing brain and optic vesicles.

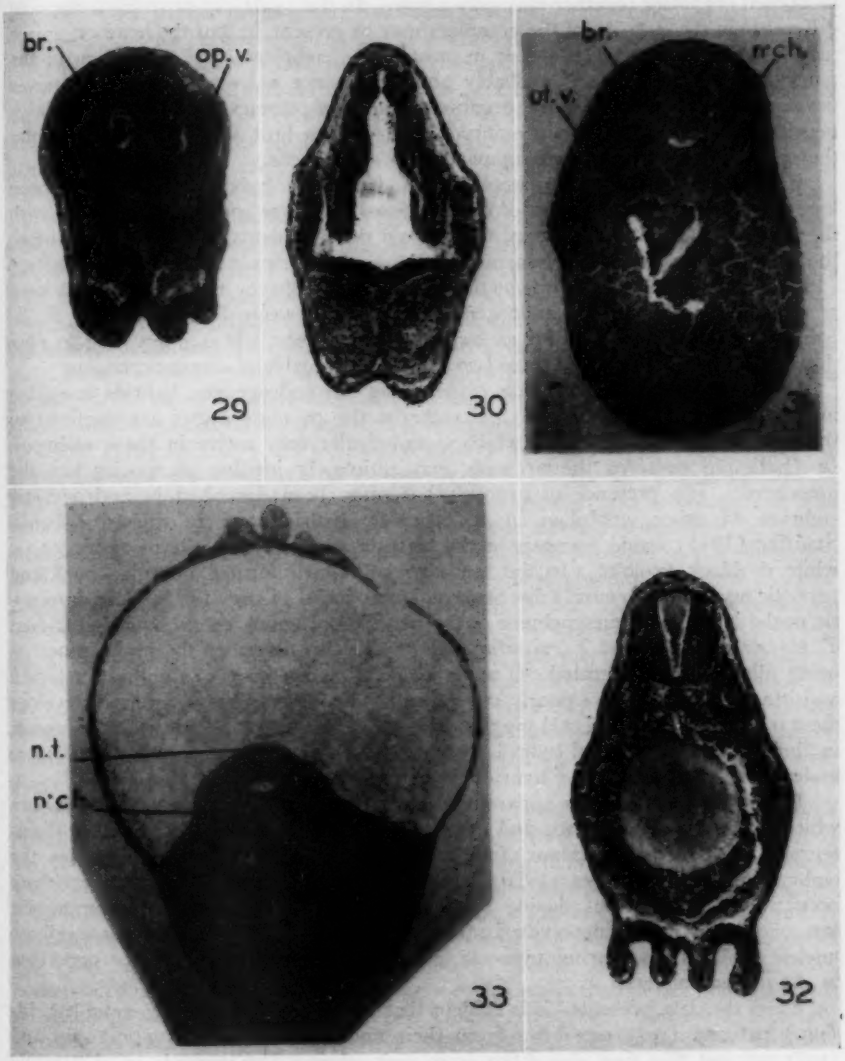
FIGURE 30. Section of embryo pip pip 613, 420 micra from anterior end, showing normal brain and optic vesicles.

FIGURE 31. Section of embryo (pal) pip 184-9, 740 micra from anterior end, showing otic vesicle.

FIGURE 32. Section of embryo pip pip 613, 800 micra from anterior end, showing normal otic vesicles.

FIGURE 33. Section of embryo (pal) pip 184-9, 2350 micra from anterior end, showing abnormal neural tube and large oedematous space.

PLATE III



ABBREVIATIONS FOR PLATES

br., brain
op. v., optic vesicle
ot. v., otic vesicle
n'ch., notochord
n. ect., neural ectoderm
n'ral, neural tissue
n'roid, neuroid tissue
n.t., neural tube

resulting in abnormal development and early death. Both lowered competence and weakened organizer together may play a role. In the (pal) pip neurulae a regional difference in the strength of the organizer may be present, in that the head organizer may be very weak or even absent in most of the embryos. Differentiation of the anterior end of the gut is probably abnormal, since a liver diverticulum never develops. Implantation of the organizer and transplantation of the presumptive neural ectoderm of (pal) pip embryos to a foreign host would clarify the roles they play in the abnormal development of these embryos.

From previous work on amphibian androgenetic hybrids it has long been known that when a haploid set of chromosomes from one species is combined with the cytoplasm of another species, the haploid development differs from that when the chromosomes and cytoplasm are of the same species. For example, Baltzer (1920) has shown that androgenetic *T. taeniatus* embryos form larvae and even reach metamorphosis, but androgenetic hybrids between *T. taeniatus* ♀ X *T. palmatus* ♂ develop only as far as larvae with forelimbs. It is interesting to note that diploid hybrids between these two species are capable of metamorphosing.

From the data presented it is evident that the androgenetic hybrids are alike in showing pycnosis. In (pip) pal embryos the pycnotic nuclei are confined to the neural ectoderm, a region which is mitotically very active in these embryos. In (pal) pip embryos the pycnosis may ultimately involve all tissues but the notochord. The presence of pycnosis has often been described in androgenetic embryos of other amphibia, in androgenetic hybrids and in diploid hybrids. Stauffer (1945) found pycnosis in the blastula and gastrula cells of androgenetic white or black axololts. In the few embryos which formed neurulae he found pycnotic nuclei in the neural tube, brain and mesoderm. Curry (1936) found pycnotic nuclei in the head mesenchyme of the neurulae of androgenetic hybrids between *T. alpestris* females and *T. cristatus* males. He also described the brain lumen as being filled with degenerated cell material. The neural tube was without a lumen and it and the brain were poorly developed. This description of internal development is similar to that of (pal) pip development. Baltzer (1938) described pycnosis in the gastrulae of diploid hybrids between *T. cristatus* females and *T. palmatus* males. Only a few of these hybrids develop as far as neurulae.

From the foregoing it is apparent that pycnosis may be characteristic of tissues which are approaching death and have ceased differentiation and begun to disintegrate. It is a manifestation of abnormal processes which are going on in the embryos. Leuchtenberger (1950) has shown that definite chemical changes are occurring in the nucleus during pycnosis. These changes involve a progessive loss of protein with a depolymerization and progressive loss of the desoxyribose nucleic acid. Thus, during pycnosis, significant chemical changes are occurring in the nuclear material.

From the data presented it is evident that not only do the androgenetic hybrids (pip) pal and (pal) pip differ from their androgenetic controls (pip) pip and (pal) pal, but that they also differ from each other. The haploid set of paternal chromosomes of either pipiens or palustris, when present in its own specific cytoplasm, is capable of normal gastrulation, neurulation and the formation of tadpoles. If pipiens cytoplasm is substituted for palustris cytoplasm, the haploid set of palustris chromosomes is only capable of carrying differentiation to the neural plate stage.

Since the genes in (pip) pal embryos are similar to those in (pal) pal embryos, the difference in the development between (pip) pal and (pal) pal is probably due to differences between pipiens and palustris cytoplasm. In (pal) pal embryos the palustris cytoplasm is able to supply normal materials to the palustris genes for their synthesis. In addition the palustris genes have the palustris cytoplasm to act upon and utilize in differentiation. In the pipiens cytoplasm certain substrates may be the same as those in the palustris cytoplasm, even as certain of their genes are homologous, since the reciprocal diploid hybrids develop normally. (Homologous spotting genes have been shown to be present in pipiens and palustris by Moore, 1943.) Other substrates in the pipiens cytoplasm may be species specific. Baltzer (1930) suggested from his studies of androgenetic hybrids that species specific substances existed in the cytoplasm. The palustris genes may not be able to utilize these species specific substrates, so that development ceases; or they may be able to utilize these substrates in forming compounds which may then act as anti-metabolites (Woolley, 1947), resulting in abnormal development. Similarly, in the reverse cross (pal) pip, the pipiens genes may not be able to utilize certain of the palustris substrates, or these substrates may act as anti-metabolites when utilized, with resulting abnormal development.

Since (pip) pal and (pal) pip embryos differ from each other in their development, different morphogenetic processes are probably upset. The uniformity of (pip) pal development suggests that a certain morphogenetic substance (or substances), which may contribute to one early major developmental step, is lacking or abnormal. For example, such a substance might be involved in the conversion of chordamesoderm to notochord. In this case one might think that the palustris gene controlling this major developmental step is not able to work with the pipiens substrate to form the necessary materials. In the reverse cross, (pal) pip, the great variation in development may indicate that several different substances which contribute to different developmental steps may be lacking or abnormal. In this case one might say that several pipiens genes may be involved in a variety of developmental steps, but are unable to work with the palustris substrates, resulting in abnormal and variable development, since a larger number of different genes and substrates are involved.

Differences between reciprocal androgenetic hybrids and their androgenetic controls have not been previously described and analyzed in detail. Baltzer (1933) has recorded differences between reciprocal androgenetic hybrids of *T. palmatus* and *T. alpestris*. Dalton (1946) noted differences between the reciprocal androgenetic hybrids and androgenetic controls among *T. torosus, T. granulosis* and *T. rivularis* embryos but this work dealt primarily with the role cytoplasmic properties may play in transplanted androgenetic hybrid tissue. Dalton found that such transplanted tissue shows the influence of the cytoplasm in the number and dispersion of melanophores. Hadorn (1936) had earlier attempted to show cytoplasmic influence in transplanted androgenetic hybrid epidermis, but with equivocal results.

The results obtained in this study suggest that the abnormal development and death of haploid amphibian embryos is due to both haploidy *per se* and to unmasked lethal and semi-lethal genes. In the development of androgenetic hybrids not only may these two factors be operative but, in addition, the cytoplasmic substrates may play a role.

Summary

The development of androgenetic *R. pipiens* and *R. palustris* embryos has been briefly described. A detailed study of their androgenetic hybrids has been made.

The (pip) pal embryos develop uniformly to an early neural fold stage, then differentiation ceases, the neural plate cells are sloughed off and the embryos die.

The (pal) pip embryos develop uniformly until early gastrulae. Thereafter their development is highly variable. Gastrulation is usually incomplete, and a short neural plate may be formed. In a few embryos closure of the neural folds may occur and neuroid and neural tissue be formed. An occasional embryo is capable of slightly further differentiation, with an abnormal brain and neural tube, and optic and otic vesicles.

The effects of haploidy and the results obtained by combining a haploid set of chromosomes of one species with the cytoplasm of a different species are discussed.

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ACTION OF ESTRONE AND PROGESTERONE ON NUCLEAR VOLUME (STUDIED BY APPLYING THE KARYOMETRIC–STATISTICAL METHOD)

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The action of hormones in tissues is demonstrated by the appearance of specific changes (hypertrophy, hyperplasia, edema, etc.) which can, under certain circumstances, be induced by different hormones.

Variations of nuclear volumes, exactly pointed out by the Karyometric-statistical method as mentioned in preceding papers (Salvatore, 1948a, 1948b), may be, in themselves, a suggestion of the action of certain hormones. Having this in mind, we decided to begin a series of researches in order to observe the action of sexual hormones on nuclear volumes, to find the maximal and minimal doses able to bring about duplication of nuclear volume in uterine cells and possibly also, to find the doses of hormone able to induce a nuclear polyploidy.

As already stated in preceding papers (Salvatore and Schreiber, 1947a; Salvatore, 1948a, 1948b), nuclei of muscular cells in female pregnant rats attain volumes 4, 6 and 8 times the size of the smallest volume found; whereas, endometrial cells have their nuclei generally remaining double in volume.

Recent Karyometric-statistical researches point to the close relationship between the nuclear volume and the number of chromosomes (genome); hence, the present study possibly may be able to show the action of chemically known substances (hormones) on the genic materials influencing their reproduction.

The Karyometric-statistical method also constitutes an excellent means of elucidating the nature of nuclear hypertrophy; for some authors claim that nuclear hypertrophy is due to trophic phenomena, while others attribute it to phenomena of a genic or chromosomic nature.

In our present report, we shall study the action of estrone and progesterone on the nuclear volume of endrometrial glands of the uterus of castrated female rats by applying the Karyometric-statistical method.

Material and Technique

We used in our present experiment, 10 full grown albino female rats (*B. Wistar*) which were submitted to ovariectomy. After one month of castration, we injected in two rats, intramuscularly, 0.5 cc. of an oily solution of estrone (Oestrone Abbott) containing 100 I.U. estrone, for 3 days, totalling 300 I.U. (record 3 and 4); in two female rats, 250 I.U. were injected per day for 4 days, totalling 1000 I.U. (records 5 and 6); two female rats with an oily solution of progesterone (Lutocyclin Ciba) in a daily dose of 0.5 mgr. for 2 days, total dose equaling 1.0 mgr. (records

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TABLE I

N° of protocol	Stages	Dose	Modal value		Nuclear size																		N° of nuclei	Mitotic index
					8.5	9	9.5	10	10.5	11	11.5	12	12.5	13	13.5	14	14.5	15	15.5	16	16.5	17	17.5	
12	Castrate	—	523		4	10	17	*47*	35	15	10	7	5											150	—
13	Castrate	—	539		1	4	10	*43*	39	29	20	4												150	—
3	Estrone treatment	300 I.U.		1150							1	2	23	*51*	42	22	7	1	1					150	1.1%
4	Estrone treatment	300 I.U.		1177								11	15	*56*	36	25	6	1						150	0.5%
5	Estrone treatment	1000 I.U.		1150								5	24	*55*	33	16	6	2	*4*	*4*	—	—	1	150	1.5%
6	Estrone treatment	1000 I.U.		1150							1	6	21	*61*	40	15	4	2						150	1.3%
7	Progesterone treat.	1 mgr.	572		2	7	22	46	53	13	7													150	—
9	Progesterone treat.	1 mgr.	523			7	27	*58*	30	20	11	6	1											150	—
10	Progesterone treat.	2 mgr.	508			6	43	*59*	28	11	8	5												150	—
11	Progesterone treat.	2 mgr.	523			7	26	*50*	29	21	8	6	3											150	—

TABLE II

Stages	Average of modal value		Average in μ^3		Nuclear size																		N° of nuclei
					8.5	9	9.5	10	10.5	11	11.5	12	12.5	13	13.5	14	14. 5	15	15.5	16	16.5	17	17.5	
Castrate	531.0		88.1μ^3		5	14	27	*90*	74	44	30	11	5											300
Estrone		1156.7		192.0μ^3							2	24	83	*223*	151	78	23	6	5	4			1	600
Progesterone	531.5		88.2μ^3		7	27	138	*213*	140	65	34	17	4											600

7 and 9); and two female rats getting 0.5 mgr. daily for 4 days, total dose equaling 2 mgr. (records 10 and 11). Two female rats (records 12 and 13) acted as controls.

After the last injection, slides of vaginal secretion, obtained from all animals, showed diestrum in castrated controls and in those injected with progesterone, and typical estrus in those injected with estrone.

The animals were killed 6 hours after the last injection. Fragments of uterine material were fixed on alcoholic Bouin and mounted on paraffin and sections measuring 10 micra were stained with hematoxylin-eosin.

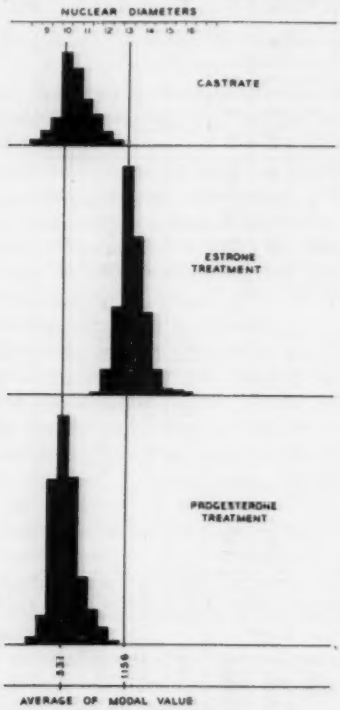

FIGURE 1. Histograms representing the frequency of nuclear volumes.

The Karyometric-statistical method was applied according to the same principle used in previous publications (Salvatore and Schreiber, 1947a, 1947b; Salvatore, 1948a, 1948b).

With a magnification of 1800 diameters, outlines were drawn by camera lucida, 150 nuclei for each sample. Arithmetical means of the diameters of nuclei were grouped into frequency classes; nuclear volumes and modal values were calculated by applying the same formulas used in preceding works (Salvatore, 1948b) and are represented in Table I, together with the mitotic indexes (number of mitoses per 100 nuclei measured).

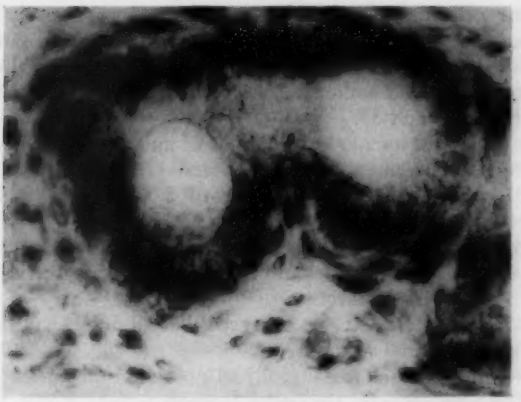

FIGURE 2. Nuclei of cells of the glands pertaining to uterus of castrated animals (control) (Prot. 12) (× 1100).

With the help of results from addition of the frequencies of the arithmetical means of nuclear diameters pertaining to samples of the castrated group, the castrated group injected with estrone, and the castrated group with progesterone (Table II), average histograms were designed, as represented in Figure 1.

Knowing that on an amplification of 1800 times, 1 mm. is equal to 0.55 micron, the nuclear volume of cubic micra was calculated and registered in Table II.

RESULTS

Analyzing Table II, one notices that in the controls as well as in the animals injected with progesterone, arithmetical means of the drawings of nuclei of endo-

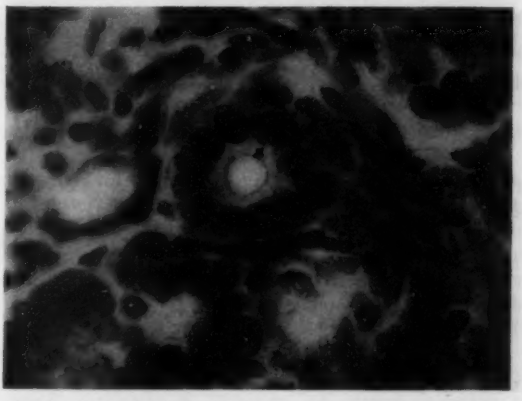

FIGURE 3. Nuclei of cells of the glands pertaining to uterus during progesterone treatment (Prot. 9) (× 1100). The size of these nuclei is the same as that of the control.

metrial glandular cells present only one maximum of frequency, represented by the modal means value of 531.0 and 531.5 or 88.1 and 88.2 cubic micra, respectively.

As noticed in Figure 1, the histogram of controls as well as of those injected with progesterone presents only a unimodal curve, thus demonstrating the homogeneity of nuclear sizes (Figs. 2 and 3).

The same is to be said for nuclear volumes of animals injected with estrone, the histogram of which is also unimodal (Figs. 1 and 4), but of a modal mean value of 1156.7 or 192.0 cubic micra. Only in record 5 (Table I) is found a general delineation of another mode.

The relationship among modes of the control groups and of those injected with estrone stands as 1:2. No intermediate modal values (1.5), in a 1:2 progression, were found as in preceding researches.

With both doses of estrone, it was observed that all nuclei had the same reaction, namely, doubling their initial volumes.

Progesterone, in the doses employed, had no action on nuclear volumes, which remained identical with those of the control group.

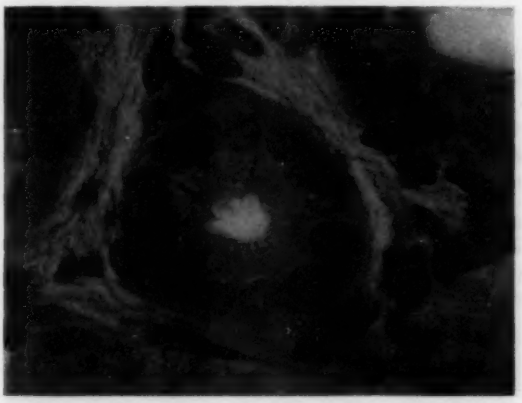

FIGURE 4. Nuclei of cells of the glands of the uterus during oestrone treatment (Prot. 5) (× 1100).

COMMENTS

The action of estrone and progesterone on uterine tissues is perfectly established by now, through works of Corner and Allen (1929), Hisaw and Leonard (1930), Clauberg (1930), Bureau (1939) and Sammartino and Nogues (1945), which allows us to state that oestrogens are responsible for phenomena of uterine growth while progesterone is responsible for changes of structures previously acted on by oestrogens.

Orville and Overholser (1938) reported a typical hypertrophy of muscular cells brought about through estrone injections on castrated female rats.

Pfeiffer and Hooker (1944) obtained nuclear hypertrophy of endometrium estrone cells in female ovariectomized monkeys, by using oestradiol benzoate, or

oestradiol benzoate and progesterone simultaneously, and also with the use of progesterone alone.

The action of progesterone on castrated animals not previously submitted to an oestrogen treatment, has already been pointed out by several authors, among them Hisaw (1935), Hisaw, Greep and Fevold (1937), Selye (1940) and Hooker (1940).

More recently, Forbes and Hooker (1947) verified that progesterone alone changes fusiform nuclei of the stroma into vesicular ones.

The results of the present report not only support the conclusion that oestrone brings about nuclear hypertrophy in endometrial cells, but also confirms the results of our researches (Salvatore and Schreiber, 1947a; Salvatore, 1948b) that hypertrophy may be perfectly evaluated by applying the Karyometric-statistical method.

The photographs of nuclei pertaining to uterine endometrial cells of animals injected with oestrone, 1100 times amplified, show a large difference of nuclear sizes (Figs. 2, 3 and 4) when compared to those of the control group. Thus it can be considered as a reliable *test* for oestrone. We have also noticed that the doses of progesterone used by us in our present experiment have no action on the nuclear volume.

The Karyometric-statistical method also established the fact that nuclear hypertrophy is essentially of a chromosomal nature and not consequent to imbibition phenomena, for the modal values are in the progression of 1:2.

Thus, all nuclei which, in the castrated animal, remain resting (basic volume, probably diploid) undergo an interphasic growth, ending by the duplication of their initial volumes.

The Karyometric researches began with Jacobj (1926) showing that physiologically the nuclei of different tissues grow "rhythmically" under the action of normal growth stimuli.

Later the rhythmic growth of nuclear volume, following a geometrical progression 1:2:4:8, was confirmed by Hertwig (1938–39), Wermel (1933), D'Ancona (1941), Sauser, Muller, Keller and Geitler (quoted by Schreiber, 1946) and Hintzsche (1945, 1946, 1949).

Dawson (1948), studying the renal tubules of the "Australian desert frog," not only noticed the existence of uninucleated (diploid unity), binucleated, trinucleated and quadrinucleated cells, but other cells with progressive nuclear sizes as well.

Besides, Jacobj (1926) noticed that during spermatogenesis, a rhythmical reduction in nuclear volumes occurs (4:2:1). The same phenomenon was noticed in the liver during development of carp (*Cyprinus carpus* var. *specularis*) by B. Schreiber and Angeletti (1940) and in liver of tadpoles belonging to *Bufo vulgaris* by G. Schreiber and M. R. Schreiber (1941).

All of these researches as well as, more recently, those of Biesela, Poyner and Painter (1942) on neoplastic tissues, and those of Schreiber (1946, 1947) on polyploid plant of coffee, as well as in ophidian spermatogenesis, clearly show the genic nature of rhythmical growth, *i.e.*, "an intimate correlation between nuclear volume and the chromosomic number."

The results of preceding researches on the nuclear growth on the endometrial and muscular cells belonging to uterine of female rats, during the oestral and gravidic cycle (Salvatore, 1948a, 1948b), during the puerperal involution (Salvatore, 1949), and in ovaric tissues (Salvatore and Schreiber, 1947b) not only confirm the results obtained by several authors but show as well that nuclear hypertrophy is of a chromosomic nature and not due to simple trophic phenomena.

The present report points out that the Karyometric-statistical method can be applied in endocrinology not only as a "test" (for it demonstrated very precisely the action of oestrogens—Oestrone—on the nuclear volumes), but also because as already stated, it clears up the real nature of nuclear hypertrophy.

Once established through cyto-genetic researches that "Duplication of nuclear volume expresses the reproduction of genes," we may insist that "Oestrone acts on the genes," inducing their reproduction.

Conclusions

1. The Karyometric-statistical method can be applied in endocrinology as a test for the determination of the action of oestrogens on uterine tissues.

2. Oestrone induces interphasic growths on nuclei pertaining to endometrial glandular cells of castrated female rats. This growth brings about a duplication of volume (1 :2).

According to cyto-genetic researches which show an intimate dependence of the nuclear volumes and number of chromosomes, it is possible that "Oestrone acts on the genes, determining their reproduction." On the other hand, this fact emphasizes the notion that nuclear hypertrophy is essentially of chromosomal nature.

3. Progesterone has no action at all on the nuclear volume of endometrial cells, which remains identical to that of the controls.

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NUTRITIONAL REQUIREMENTS OF THE EARLY CHICK EMBRYO. III. THE METABOLIC BASIS OF MORPHOGENESIS AND DIFFERENTIATION AS REVEALED BY THE USE OF INHIBITORS [1]

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Introduction

In an attempt to learn something about the metabolic changes underlying the developmental processes of morphogenesis and histogenesis, a study of the nutritional requirements of the embryo was begun several years ago (Spratt, 1947). It was thought that a better understanding of the control mechanisms which characterize the differentiation process could be obtained by an examination and comparison of the nutritional properties of differentiating cells with "undifferentiated" and already differentiated cells. Subsequent studies have shown that both initiation and maintenance of particular cellular movements and differentiations of the early chick embryo are dependent upon an adequate nutrient environment, and that different developmental activities have both quantitatively and qualitatively different environmental requirements (Spratt, 1948a, 1948b, 1949a, and 1950). Furthermore, it was shown that differences in metabolism of the completed organs and tissues of the adult are already present during their development.

A further analysis of the biochemical reactions underlying the various developmental activities has been attempted by the use of metabolic inhibitors, substances which have been shown to block or interfere with enzyme activity. Differences in the effects of inhibitors on different embryonic processes would, presumably, indicate differences in the metabolic pathways being used.

Correlation of the effects of inhibitors with those produced by several other environmental changes on particular developmental processes may enable us to construct a better over-all picture of the metabolic basis of embryonic development, even though many important details are lacking. Evidence to be presented, coupled with that provided by preceeding studies, enables us to make the following tentative generalization.

Regions of the chick embryo where cells are undergoing active and rapid differentiation (*e.g.*, the node, the fore-brain, etc.) are more dependent upon an adequate nutrient environment than are regions already differentiated or "undifferentiated." In other words, the pattern of metabolic (enzymatic) activity seems to be directly related to the pattern of differentiation activity.

[1] Aided by a grant from the National Institutes of Health, U. S. Public Health Service.

[2] I am grateful for the excellent facilities provided by The Johns Hopkins University where this investigation was done.

Experimental Methods

Approximately 600 chick blastoderms (definitive primitive streak to 8-somite stages) were removed from incubated eggs and explanted to the surface of synthetic media *in vitro*. Operative techniques, culture methods and methods of preparing media were the same as those previously described (Spratt, 1947, 1948a and 1949a). Compositions of the various control and experimental media will be given below. Results have been recorded in the form of camera lucida drawings of the living explants, made by the author. Most of the explants were dissected under saline; a few were fixed, stained and sectioned for study of histological details.

Procedure and Results

In studying the effects of inhibitors on early development, two types of control series were set up for each experiment: (1) embryos explanted to non-nutrient, Ringer media and (2) embryos explanted to glucose-Ringer media. An additional type of control series was set up for each experiment on the effects of monoiodoacetate and sodium fluoride: (3) embryos explanted to sodium pyruvate-Ringer media.

A. Development on Control Media

(1) Non-nutrient, Ringer medium. The medium has the following composition, expressed in grams per 100 ml. of medium: NaCl 0.9, KCl 0.042, $CaCl_2$ 0.024, $Na_2HPO_4 \cdot 12H_2O$ 0.0145, KH_2PO_4 0.0026, $NaHCO_3$ 0.055, Agar 0.35.

Explants to the non-nutrient medium begin to degenerate after an initial lag period (2–4 hrs.). The characteristic feature of the degenerative process is a breakdown of structural organization at the organ and tissue levels and dispersal of the cells (Spratt, 1947, 1948a and 1949a). The spatial pattern and time course of the degenerative process is illustrated in Table I. The region most sensitive to extreme starvation is the node, the center of most active morphogenesis and histogenesis during the first two days' development. A second region of high susceptibility is the anterior end of the embryonic axis, the head-fold, where foregut, fore-brain and optic vesicle formation occur.

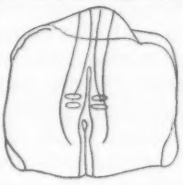
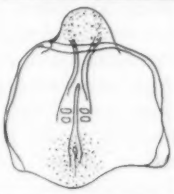
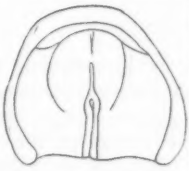
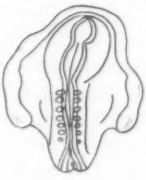
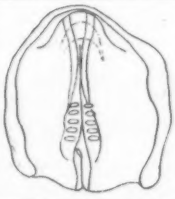
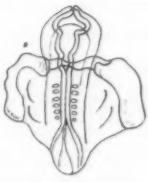
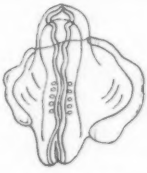
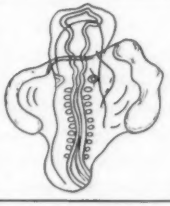
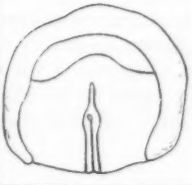
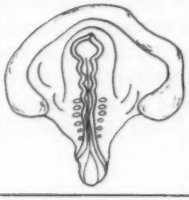
(2) Glucose-Ringer medium. This is simply the non-nutrient medium containing 10^{-2} to $5 \times 10^{-2} M$ d-glucose. In most instances, $10^{-2} M$ glucose was used.

Explants to the glucose medium undergo morphogenesis and differentiation as illustrated by the typical example in Table I: (a) regression of the primitive streak, formation and elongation of the notochord, (b) formation of the head-fold and neural plate, (c) development of the brain, spinal cord, optic vesicles, otocysts and somites, (d) formation and pulsation of the heart, etc. In the absence of an exogenous nitrogen source, the extent of histogenesis is inferior to that obtained in embryos explanted to yolk-albumen media (Spratt, 1947).

The effects of partial carbohydrate starvation (accomplished by decreasing the glucose concentration or by using a less efficiently utilized sugar) were again observed. The results confirm those previously reported (Spratt, 1949a and 1950) in showing that the embryonic region most sensitive to the nutritionally poor environment is the node, with the head-fold region the next most sensitive.

TABLE I

Control series

MEDIUM	NO. EXPL	STAGE EXPLANTED	GENERALIZED RESULTS	
			10± HOURS IN VITRO	20± HOURS IN VITRO
RINGER-BUFFER	56			
RINGER-BUFFER GLUCOSE 10^{-3}–5×10^{-2}M	140			
RINGER-BUFFER PYRUVATE 10^{-1}–4×10^{-1}M	105			
RINGER-BUFFER LACTATE 2×10^{-2}M	32			
RINGER-BUFFER PYRUVATE 2×10^{-2}M + GLUCOSE 10^{-3}M	28			
RINGER-BUFFER PYRUVATE 2×10^{-2}M + GLUCOSE 2.5×10^{-4}M	4			

(3) Pyruvate-Ringer medium. The non-nutrient medium containing 10^{-2} to $4 \times 10^{-2}M$ sodium pyruvate. In most instances, $2 \times 10^{-2}M$ was the concentration used.

The type of development which occurs on the pyruvate control medium is illustrated in Table I. Although pyruvate can be utilized for supporting some of the early developmental changes, it is inferior to glucose (Spratt, 1949b and 1950). After the first 15–20 hours' cultivation practically all further development ceases and the embryos shrink to an appreciably smaller size than the corresponding glucose controls. Histogenesis of the brain and heart is also inferior. Development on sodium lactate media ($2 \times 10^{-2}M$) is approximately equivalent to that on pyruvate. Some observations indicate that lactate is slightly superior (Table I) but this needs further study.

B. Development on Media Containing Combinations of Glucose and Pyruvate or Lactate

Seven experiments in which a sub-minimal amount of glucose ($2.5 \times 10^{-4}M$) was added to either a minimal ($5 \times 10^{-3}M$) or optimal ($2 \times 10^{-2}M$) pyruvate medium were done.[3]

Results of two experiments are shown in Table I. Here both minmial ($10^{-3}M$) and sub-minimal ($2.5 \times 10^{-4}M$) amounts of glucose plus $2 \times 10^{-2}M$ pyruvate result in development indistinguishable from that of the glucose (10^{-3} to $10^{-2}M$) controls. In general, when both glucose and pyruvate (even at molar concentration ratios of 1:20 or 1:80, respectively) are present in the medium, the embryos are similar in their extent of development to those utilizing glucose alone—not dwarfed like the pyruvate controls. Combinations of sodium lactate and glucose have yielded similar results.

C. Metabolic Inhibitor Effects

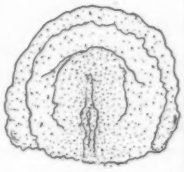
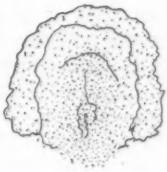
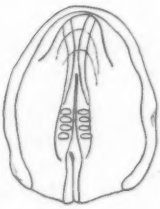
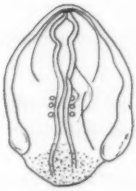
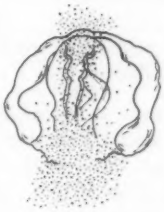
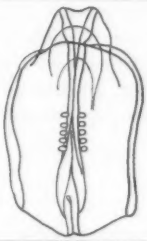
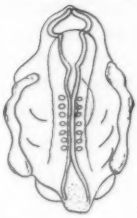
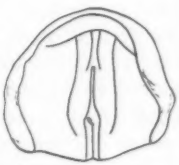
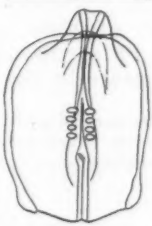
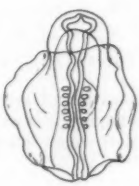
(1) Monoiodoacetate (sodium salt). In these and all other inhibitor experiments, a measured amount of a sterilized stock solution of the inhibitor was added to the medium after it had been autoclaved and cooled to about 40° C. The pH of the medium was adjusted to 7.5–8.5 with NaOH (8.0 in most experiments). The pH was measured with the Beckman pH meter at the beginning and end of each experiment and found to remain constant in almost all instances.

The results obtained are summarized in Table II. It is to be noted that $10^{-4}M$ iodoacetate produces almost immediate stoppage of development and complete degeneration, dispersal and disintegration of the entire embryo. The degree of inhibition at this concentration compared with the type of degeneration resulting from substrate deprivation (non-nutrient controls), indicates that the utilization of endogenous reserves as well as of exogenous substrates is blocked. At lower inhibitor concentrations ($5 \times 10^{-5}M$ and $2 \times 10^{-5}M$) the effects are less drastic and become differential (Table II). It is noteworthy that the time course and

[3] It is possible that the utilization of an excess amount of pyruvate might "throw out of gear" the entire metabolic mechanism by leading to the accumulation of excess endogenous metabolites such as ATP. Glucose might act as a phosphate acceptor and prevent its accumulation (Ochoa, 1941).

TABLE II

Monoiodoacetate

MEDIUM	NO. EXPL	STAGE EXPLANTED	GENERALIZED RESULTS	
			10± HOURS IN VITRO	20± HOURS IN VITRO
GLUCOSE 10^{-2}M + CH_2ICOOH 10^{-4}M	10			
GLUCOSE 10^{-2}M + CH_2ICOOH 5×10^{-5}M	26			
GLUCOSE 10^{-2}M + CH_2ICOOH 2×10^{-5}M	26			
PYRUVATE 2×10^{-2}M + CH_2ICOOH 10^{-4}M	9			
PYRUVATE 2×10^{-2}M + CH_2ICOOH 5×10^{-5}M	17			

spatial pattern of the differential degenerative process is almost identical with that occurring in the non-nutrient controls: the node and the head-fold, in this order, being the two most sensitive regions. In other words, actively differentiating regions seem to be particularly sensitive to the inhibitor. Eventually the entire neural axis (brain and cord) undergoes degeneration whereas the heart is relatively unaffected, continuing to develop and pulsate (Spratt, 1949a). The fact that the heart will develop in primitive streak blastoderms, in the presence of the inhibitor, argues against the possibility that the differential effect reflects primarily differences in the extent of development of the heart compared with the brain, *i.e.*, not only the maintenance but the initiation of heart-formation is more resistant than the brain. These differential effects on the processes of brain and heart-formation revealed by using the lower concentrations of iodoacetate suggest that the underlying metabolic processes are different.

Further elucidation of the apparently different metabolic mechanisms underlying brain-formation as compared with heart-formation would depend, partly, upon evidence as to the point of action of iodoacetate on the metabolic pathway.[4] The fact that both complete and differential inhibition of development by iodoacetate can be prevented by substitution of sodium pyruvate for glucose in the culture medium (Table II) suggests that the site of action of the inhibitor in the chick embryo is similar to that described for other organisms, namely, that it interferes with triosephosphate dehydrogenase. Partial protection against the effects of $10^{-4}M$ iodoacetate and complete protection against $5 \times 10^{-5}M$ iodoacetate are obtained with $2 \times 10^{-2}M$ pyruvate. The more extensive degeneration of the developing nervous system in the presence of glucose and iodoacetate (which presumably interferes with the important oxidative-phosphorylation reaction) [4] suggests its greater dependence upon oxidative metabolism as compared with the heart. Partial prevention, with pyruvate, of the complete degeneration produced by $10^{-4}M$ iodoacetate exhibits a differential pattern of degeneration which is correlated with the regional pattern of developmental activity (Table II).

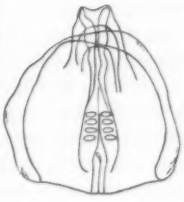
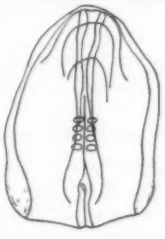
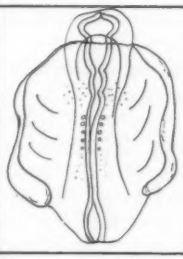
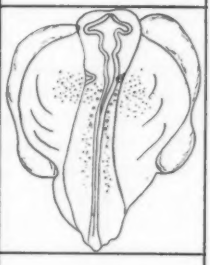
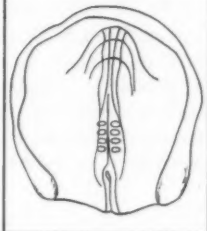
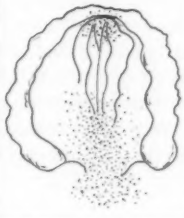
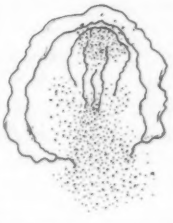
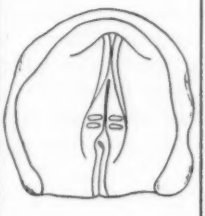
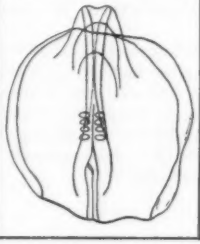
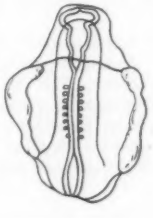
(*2*) *Sodium fluoride*. The effects of this inhibitor and their prevention by substitution of pyruvate for glucose are summarized in Table III. Explants to a glucose-Ringer medium containing $10^{-2}M$ sodium fluoride undergo differential and then complete degeneration, the regional pattern of which is comparable to that resulting from substrate deprivation (Tables I and III). This is in contrast to $10^{-4}M$ iodoacetate which produces immediate and complete degeneration (Table II). Fluoride, at this concentration, apparently does not extensively inhibit the utilization of endogenous reserves.

Lower concentrations of fluoride ($5 \times 10^{-3}M$) result in a differential inhibition of developmental processes which is almost the converse of that obtained with iodoacetate: The heart fails to develop or degenerates if already forming at the

[4] It is generally held that small amounts of iodoacetate (3×10^{-4} to $3 \times 10^{-3}M$) inhibit triosephosphate dehydrogenase, the enzyme catalyzing the reaction: 1, 3 Diphosphoglyceraldehyde $\rightleftarrows$ 1,3 Diphosphoglyceric acid (Dixon, 1937; Adler, v. Euler and Günther, 1938; Rapkine, 1933 and 1938). Originally it was thought that iodoacetate completely blocked anaerobic glycolysis in concentrations which had little or no effect on respiration (Lunsgard, 1930) but subsequently it has been shown that these concentrations ($3 \times 10^{-4}M$) also markedly depress respiration in rat brain and testis (Krebs, 1931; Fuhrman and Field, 1943).

TABLE III

Sodium fluoride

MEDIUM	NO. EXPL	STAGE EXPLANTED	GENERALIZED RESULTS	
			10 ± HOURS IN VITRO	20 ± HOURS IN VITRO
GLUCOSE 10^{-2}M + FLUORIDE 10^{-2}M	37			
GLUCOSE 10^{-2}M + FLUORIDE 5×10^{-3}M	44			
PYRUVATE 2×10^{-2}M + FLUORIDE 2×10^{-2}M	8			
PYRUVATE 10^{-2}M + FLUORIDE 10^{-2}M	7			
PYRUVATE 2×10^{-2}M + FLUORIDE 10^{-2}M	16			

time of explantation, whereas the central nervous system continues its development and shows little if any harmful effect of the fluoride. At lower fluoride concentrations (2.5×10^{-3} and $10^{-3}M$) there is progressively less effect upon heart-formation. The typical picture of the differential effect is that of an apparently normal embryo with a mass of dispersed and degenerating mesodermal cells occupying the position in which the heart would have formed. The degenerative effect of fluoride is not limited to heart mesoderm but involves, to some extent, the somite and lateral plate mesoderm. In a number of cases, posterior development of the neural tube is indirectly inhibited, presumably by the absence of inducing mesoderm. Some of these effects are illustrated in Figure 1.

Interpretation of the differential effects of fluoride, in terms of interference with the metabolic mechanisms underlying heart as compared with brain-formation, is largely dependent upon knowledge of its site of action.[5] The fact that complete inhibition of development by $10^{-2}M$ fluoride can be prevented by substitution of a

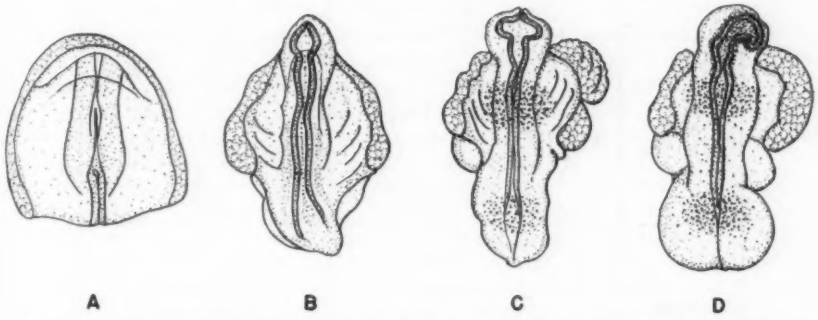


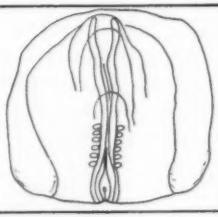
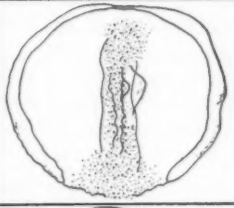
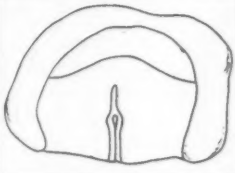
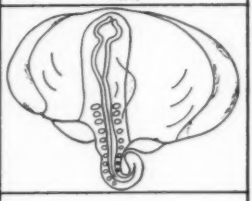
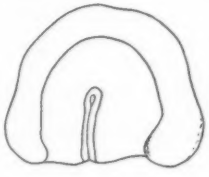
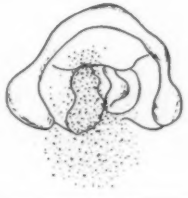
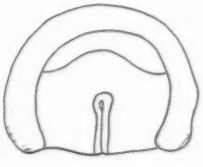
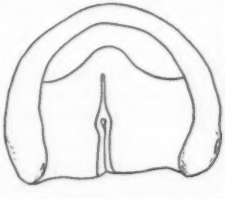
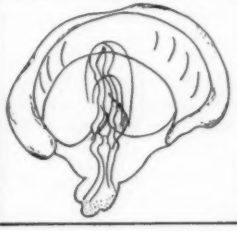
FIGURE 1. Camera lucida drawings of the anterior half of a living explant during its development on a medium containing Ringer, $10^{-2}M$ glucose, and $5 \times 10^{-3}M$ sodium fluoride. A. Stage explanted; B. 8 hours later; C. 18 hours after explantation; D. 38 hours after explantation. Note the degenerating heart mesoderm.

sufficient amount ($2 \times 10^{-2}M$) of pyruvate for glucose (Table III) suggests that the inhibitor is blocking enolase activity. If this is true, the greater sensitivity of the heart as compared with the developing brain would indicate its greater dependence upon a glycolytic energy source. Since the effect of fluoride on respiration is relatively indirect and slight (see Gemmill, 1939, for discussion of the mechanism of fluoride inhibition) compared with the effect of iodoacetate, one would expect that its interference with the metabolic basis of brain-formation would be correspondingly less drastic. Eventually, the piling up of metabolic intermediates in the presence of fluoride would presumably interfere with the glycolytic and oxidative pathways above pyruvate. Indeed, fluoride does have a delayed inhibitory effect upon the nervous system during the second day of cultivation (Fig. 1).

[5] It is generally held that fluoride inhibits the enzyme enolase which catalyzes the glycolytic reaction: 2-Phosphoglyceric acid $\rightleftarrows$ Phosphoenolpyruvic acid, by formation of a magesium-fluoride phosphate complex (Meyerhof and Kiessling, 1935; Warburg and Christian, 1942).

TABLE IV

Citrate, malonate, cyanide, azide
($10^{-2}M$ glucose present in all media)

INHIBITOR	NO. EXPL	STAGE EXPLANTED	GENERALIZED RESULTS 20± HOURS IN VITRO
CITRATE 5×10^{-3}M	29		
MALONATE 10^{-3} M	38		
CYANIDE 5×10^{-3}M	13		
CYANIDE 10^{-2}M	12		
AZIDE 5×10^{-4}M	6		
AZIDE 10^{-4}M	6		

(3) Sodium citrate. Not only is the early embryo unable to utilize citrate as a carbon source but its utilization of glucose is partially inhibited by 5×10^{-3} to $10^{-2}M$ concentrations of sodium citrate (Table IV). It was thought that citrate would produce an inhibitory effect like that of fluoride because of the formation of relatively insoluble magnesium citrate which would displace magnesium, the normal activator of enolase. However, since there are several other enzymes which depend upon magnesium as an activator, only rather general inhibitory effects could be expected. In fact, citrate produces a pattern of differential inhibition similar to that obtained with iodoacetate, malonate, cyanide and azide; development of the central nervous system is inhibited, but not that of the heart.

(4) Sodium malonate. The effects of $10^{-3}M$ sodium malonate are summarized in Table IV. The differential effects produced by this and by twice this concentration are striking; usually no effect of the inhibitor is observed during the first 20 hours *in vitro*. During the next 20 hour period, the central nervous system degenerates completely. Meanwhile the heart forms and functions. The differential effect of malonate is of the same type as that produced by iodoacetate but is still more pronounced, an interesting observation since malonate is a rather specific inhibitor of succinic dehydrogenase (Quastel and Wooldridge, 1928; Ochoa, 1944) in the cyclophorase system and would not be expected to interfere directly with the glycolysis of glucose by the heart. Iodoacetate would presumably not produce as clear cut a separation of anaerobic glycolysis and respiration (see above).

If the effect of malonate on the developing embryo is the result of its competitive inhibition of succinic dehydrogenase, the addition of succinate to the medium should diminish or prevent the effect. Preliminary experiments in which both $2 \times 10^{-3}M$ malonate and $10^{-3}M$ succinate are present in the medium indicate a partial prevention of the malonate inhibition. Greater concentrations of succinate ($4 \times 10^{-3}M$) are more effective. Preliminary incubation for 6 hours in the presence of glucose and $4 \times 10^{-3}M$ succinate, followed by subculture to a medium containing glucose, malonate ($2 \times 10^{-3}M$) and succinate ($4 \times 10^{-3}M$), results in the best (incomplete) protection against the malonate effect. Attempts to by-pass the inhibited step with sodium fumarate have failed, presumably because exogenous fumarate cannot be utilized by the embryo (Spratt, 1949b and 1950).

(5) Sodium cyanide. Since it had previously been shown that oxygen deficiency has a more pronounced effect on development of the central nervous system than on the heart (Spratt, 1948b and 1950) it was of some interest to study the effect of the respiratory inhibitors, cyanide and azide, both of which according to Keilin and Hartree (1939) act on cytochrome oxidase. Reference to Table IV shows that $5 \times 10^{-3}M$ sodium cyanide in the medium (pH 7.8–8.5) has no visible effect on development. Higher concentrations ($10^{-2}M$) give the typical pattern of differential inhibition as regards the time course and spatial pattern of the degenerative process. As would be expected, the developing brain is more sensitive to cyanide than is the heart. This result is comparable with the effects of cyanide on the squid embryo (Marvel and Fisher, 1948).

(6) Sodium azide. Relatively low concentrations of sodium azide ($5 \times 10^{-4}M$) bring about immediate and complete degeneration and disintegration of the explants

(Table IV). As was the case with monoiodoacetate (see above) this concentration of azide apparently blocks the utilization of endogenous as well as exogenous substrates.[6]

Lower concentrations of azide ($10^{-4}M$) are less toxic and tend to produce a pattern of differential degeneration of the same type as that produced by iodoacetate and malonate, but never as distinct. Indeed, the results to date have shown no clear cut differential effects of azide: concentrations which are sufficiently high to bring about degeneration of the central nervous system also seem to inhibit heart-formation; those just low enough to permit heart development result in abnormal but not inhibited brain development (Table IV).

The absence of distinct differential effects of sodium azide is interesting in the light of its action as an inhibitor of synthetic processes (Clifton, 1937; Meyerhof, 1945). In the frog, azide (in contrast to cyanide) also brings about an immediate cessation of development at all stages studied (Spiegelman and Moog, 1945). Whereas some developmental changes, *e.g.*, heart-formation, can occur under partial anaerobiosis (low oxygen tension or cyanide) these cannot occur in the presence of azide, apparently, because of its additional effect upon the coupling between energy yielding and energy requiring reactions. Evidence for such an action of azide is found in the studies of Barth and Jaeger (1947) on phosphorylation in the frog's egg.

(7) Interpretation of differential effects of monoiodoacetate and sodium fluoride. The observation that mesodermal derivatives are more sensitive to fluoride than are ectodermal derivatives, together with the converse observation that ectodermal derivatives are more sensitive to monoiodoacetate than are mesodermal derivatives, indicates differences in the metabolic processes underlying the formation and maintenance of the two different germ layer derivatives. Although the exact nature of the biochemical differences is not revealed by the above observations, the following explanation is offered as a working hypothesis:

a. It has been shown that the brain is more sensitive to oxygen deficiency than is the heart (Spratt, 1948b and 1950).

b. The brain is also more sensitive to cyanide and azide, substances which interfer with cytochrome oxidase activity.

c. The brain is more sensitive to iodoacetate but the heart is more sensitive to fluoride.

d. The interpretation that the concentration of iodoacetate used ($2 \times 10^{-5}M$) is partially blocking both anaerobic glycolysis and respiration (Krebs, 1931; Fuhrman and Field, 1943), and the concentration of fluoride used ($5 \times 10^{-3}M$) is primarily inhibiting glycolysis, is consistent with the hypothesis that the brain depends primarily upon oxidative metabolism, the heart upon glycolysis.

[6] The similarity in the effects of these two inhibitors is interesting in view of the evidence (Spiegelman, Kamen and Sussman, 1948) that azide not only inhibits the Warburg-Keilin System but also the coupling mechanism between oxidation and phosphate esterification in the reaction: 3-Phosphoglyceraldehyde $\leftrightarrows$ 1,3-Diphosphoglyceric acid, *i.e.*, the diphosphoglycerate-transphosphorylase system close to the site of action of iodoacetate.

Discussion

Changes in the environment of the explanted, early embryo whether consisting of (a) the presence of metabolic inhibitors, (b) substrate deprivation (Spratt, 1949a and 1950), (c) partial anaerobiosis (Spratt, 1948b), (d) carbon dioxide deficiency (Spratt, 1949c) or (e) low pH of the medium (Spratt, 1950) all lead to a characteristic pattern of differential inhibition of development, the time course and spatial features of which are essentially identical with the axial gradient pattern of physiological activity of the blastoderm (Child, 1925; Hyman, 1927; Rulon, 1935). The common effects of these diverse environmental modifications are summarized diagrammatically in Figure 2 in terms of regional and gradient differences in sensitivity (density of stippling) of A, head-fold and B, 10-somite blastoderms. Centers of developmental activity (morphogenesis and differentiation) at the two stages are indicated by the symbols X and Y, and the specific developmental events

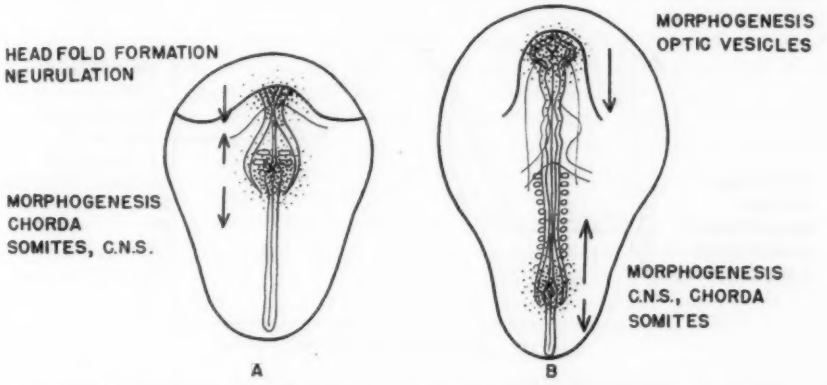


Figure 2. Diagrams summarizing the time course and spatial features of the characteristic pattern of differential inhibition of development produced by inhibitors, starvation, partial anaerobiosis, etc. Note that this pattern is correlated with that of differentiation activity. See text for further descripion.

are noted at the side. The arrows indicate the time course (spread) of the degeneration.

All of the environmental modifications have one primary effect in common: they interfere with the utilization of exogenous substrates upon which the embryo is dependent. In other words, they all retard or inhibit the metabolic processes underlying developmental activities. Furthermore, they all reveal both qualitative and quantitative differences in the nutritional requirements for formation and maintenance of different organs and tissues (Spratt, 1950). On the other hand, each environmental modification, in general, is like the others in demonstrating that the nutritional requirements for the *maintenance* of a differentiated structure (*e.g.*, brain, heart, etc.) are qualitatively the same as those for its *formation* (differentiation). These observations, together with the results of the inhibitor ex-

periments in particular, indicate that there is no qualitative difference between the metabolic basis (at least as regards the fundamental energy-yielding mechanisms) of the differentiation as compared with the maintenance of a structure. The theoretical implications of this concept of the "identity of maintenance and morphogenetic energy" have been discussed by Spiegelman (1945) in connection with his physiological competition hypothesis.

Finally, analysis of the inhibitor and previous experiments on differential nutrient requirements, in so far as they reveal a pattern of both quantitative and qualitative differences in metabolic activity in the early embryo, demonstrates that there is an apparently significant coincidence between the time course and spatial pattern of degeneration produced by inhibitors or starvation and the time course and spatial pattern of differentiation activity (Fig. 2). Regions of the early embryo where differentiation activity is greater (*e.g.*, the node and head-fold, or later, the fore-brain and the segmental plate, etc.) are more sensitive to adverse environmental conditions (presence of metabolic inhibitors,

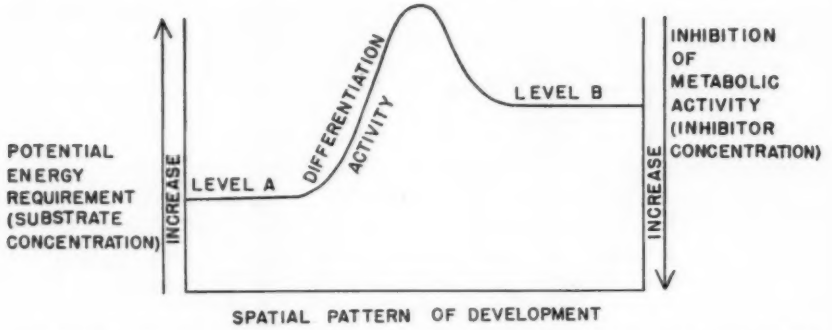


FIGURE 3. Diagram illustrating the relationship of substrate requirements and inhibitor effects to differentiation activity and to a spatial pattern of development. See text for further description.

starvation, oxygen deficiency) than are regions which have already passed through a period of differentiation activity (*e.g.*, hind-brain, spinal cord, somites, pulsating heart, etc.). It is thus not surprising that the energy requirements (in terms of substrate level necessary) for morphogenesis and histogenesis are greater than are those for maintenance. The building up of a new level of structural complexity which characterizes the form-building and tissue-building aspects of differentiation would obviously involve the expenditure of more energy than that needed to maintain the preceeding level of complexity against the ever present tendency toward "structural death."

A more thorough examination of the data pertaining to the differential effects of substrate deprivation, inhibitors, etc. reveals the extremely interesting fact that the energy necessary for the maintenance of an actively differentiating region, transforming from one level of structural complexity to the next, is *more* than the sum of the energy necessary for maintenance of each level. Thus, about 5 mg. glucose, 10 mg. fructose, or 20 mg. galactose (per 100 ml. medium) will maintain relatively

undifferentiated regions; whereas, roughly 10 mg. glucose, 20 mg. fructose or 100 mg. galactose will prevent degeneration of differentiated regions; but 15–20 mg. glucose, 50 mg. fructose or 200 + mg. galactose are necessary to maintain actively differentiating regions (*e.g.*, the node or fore-brain). Although the quantities as stated are only approximations, they are comparatively significant (Spratt, 1949a, pp. 286–291 and 1950). This extra energy requirement may be grossly compared with the "activation-energy" of a chemical reaction except, of course, that there is an *increase* in free energy of the system. In Figure 3 these relations are diagrammatically represented.

For example, as the substrate concentration in the medium is increased it will first satisfy the energy requirements of relatively undifferentiated regions, Level A (*e.g.*, extra-embryonic region compared with embryonic area, spinal cord compared with brain, etc.) and other parts of the blastoderm (both differentiating and differentiated regions) will degenerate and undergo cell dispersal. Further increase would maintain structural Level B, but not the region undergoing the transformation, A → B (*e.g.*, the node). Inhibition of metabolic activity has the converse effect. In terms of synthetic activities, the change from A → B is an increase in rate, surpassing even the rate required to maintain Level B. The greater substrate requirement and sensitivity to inhibitors of an embryonic region undergoing active transformation (*e.g.*, the node) may be compared with its greater differentiation potential [7] and array of tissue-forming potencies. It is tempting to speculate that the structural complexity of such a region is *less* than that of either a relatively undifferentiated or differentiated region.

In conclusion, it would be interesting to interpret the above relations in terms of the underlying enzymatic activities. As an hypothesis, it is suggested that enzymatic activity is inversely proportional to the level of structural differentiation or, conversely, directly proportional to the nutritional (potential energy) requirements of the embryonic region. During the differentiation process (Fig. 3) there would first be an increase in enzyme activity (associated with increased synthesis) followed by a decrease to a new level which, however, is higher than that preceeding the transformation process. The increase followed by a decrease in activity might be proportional to the release from, followed by the incorporation of enzyme proteins into, the structural organization of the cell or cell groups. It is interesting that such rapidly transforming regions as the node and fore-brain also exhibit the greatest capacity for regulation and regeneration (Waddington, 1932; Spratt, 1940). These regions, in contrast to others, might have more "free" enzymes available for the additional catalyses necessary to replace a lost part. Unpublished studies on the reduction of vital dyes, some of the studies of Child and his students, and studies of specific dehydrogenase activities now in progress at least suggest the plausibility of such a concept.

Summary

1. The effects of metabolic inhibitors on the development *in vitro* of approximately 600 chick blastoderms (definitive primitive streak through 8-somite stages) have been studied.

[7] Used in the sense as defined and described by Spiegelman (1945).

2. Embryos explanted to glucose media containing either 10^{-4} to $5 \times 10^{-5} M$ monoiodoacetate or $10^{-2} M$ fluoride rapidly undergo complete degeneration and disintegration. These effects are reversible by substitution of $2 \times 10^{-2} M$ pyruvate (or lactate) for the glucose. At lower inhibitor concentrations of iodoacetate ($2 \times 10^{-5} M$) the central nervous system degenerates or fails to form, but the heart develops and pulsates. Fluoride has almost the opposite effect: concentrations which cause degeneration of the heart ($5 \times 10^{-3} M$) have no appreciable effect upon the developing central nervous system.

3. Other inhibitors: citrate, malonate, cyanide and azide, produce a differential pattern of inhibition similar to that produced by iodoacetate. Azide, however, does not lead to as clear cut a pattern of differential degeneration as do the others.

4. Comparison of the effects of inhibitors with those produced by substrate deprivation, low oxygen tension, etc. reveals that all of these environmental modifications give rise to a characteristic pattern of differential degeneration in the blastoderm, the time course and spatial pattern of which is essentially identical with that occurring in the non-nutrient control series; the node and the head-fold, in this order, being the two most sensitive regions.

5. The pattern of differential inhibition of development produced by the presence of inhibitors or other environmental modifications coincides with the pattern of differentiation activity and, presumably, with the pattern of underlying metabolic (enzymatic) activity.

6. Analysis of the experimental results has led to the formulation of a general hypothesis relating developmental activity, energy requirements and enzyme activity to the gradual transformation from one level of structural complexity to the next.

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FURTHER OBSERVATIONS ON CERCARIA PARVICAUDATA STUNKARD AND SHAW, 1931

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In a report on the effect of dilution of sea-water on the activity and longevity of certain marine cercariae, Stunkard and Shaw (1931) described two new species of cercariae. One of them, *Cercaria sensifera*, was shown by Stunkard and Cable (1932) to be the larval stage of *Parorchis avitus* Linton, 1914 [= *P. acanthus* (Nicoll, 1906) Nicoll, 1907]. The life history and adult stages of the other species, *Cercaria parvicaudata*, is yet unknown. The original account of *C. parvicaudata* was brief, incomplete and in certain respects inaccurate; further study permits a more adequate description of the species and may aid in the elucidation of the life cycle.

Cercaria parvicaudata occurs in *Littorina littorea* (Linn., 1758) Férussac, 1822; in *L. saxatilis* (Olivi, 1792) Johnston, 1841 [= *L. rudis* (Maton, 1797) Forbes, 1838]; and in *L. obtusata* (Linn., 1758) Férussac, 1822 [= *L. palliata* (Say, 1822)].[1] So far, the infection has been reported only in the region of Woods Hole, Mass. About one per cent of the individuals of the first two species is infected, but infections in *L. obtusata* are rare. Shedding of the cercariae is irregular; often an infected snail will not shed any cercariae for several days. The larvae may emerge at night or during the day. Multiple infections with *Cercaria parvicaudata* and other species, *viz.*, cercariae of *Cryptocotyle lingua*, and with a small undescribed microphallid species, have been found in *L. littorea* and in *L. saxatilis*.

The cercariae are produced in orange-colored sporocysts, localized usually in a single, oval mass situated between the intestine and the body wall of the snail and about midway between the ends of the visceral portion. The mass may be from 2 to 10 mm. long; when large it causes protrusion of the body wall and is easily recognized by the orange color when the shell of the snail is removed. Occasionally more than one mass is present and as many as four have been observed. Each mass contains from hundreds to thousands of daughter sporocysts restricted to a circumscribed region; they do not occur in the lymph spaces throughout the body of the snail and are absent from the digestive gland, although cercariae have been found in the hemocoele when snails were dissected. It is probable that each mass of sporocysts constitutes the progeny of a single miracidium, and when two or more masses are present, which is rare, that multiple infection has occurred. The location of the sporocysts suggests that the snail ingests the embryonated egg of the trematode, that the miracidium emerges in the intestine, migrates through the

[1] Synonymy after Bequaert (1943), "The Genus *Littorina* in the Western Atlantic." In Johnsonia; No. 7, 28 pp., Museum Comp. Zool., Harvard University.

gut wall and transforms into a primary sporocyst in which asexual reproduction produces the mass of daughter sporocysts.

A half-grown specimen of *L. littorea* infected with *C. parvicaudata* and a somewhat more mature specimen of *L. saxatilis* infected with both *C. parvicaudata* and the undescribed microphallid species were killed, fixed, and cut in serial sections, 15 microns thick. Sections were stained with Ehrlich's haematoxylin and erythrosin. The specimen of *L. littorea* had been isolated for 23 days in a finger bowl. Early in the period it had shed cercariae in considerable numbers, several hundred in 24 hours, but the number gradually diminished and in the last week no cercariae were observed. Perhaps lack of food had so depleted the nutrient material in the blood of the snail that development and liberation of cercariae had stopped. One mass of sporocysts was present; at that level the intestine was pressed against the body wall and all the space to the opposite body wall was filled with sporocysts; in one section 286 sporocysts were counted. The lobes of the digestive gland were almost completely excluded from this portion of the snail and no gonad was recognized. In much of the mass the sporocysts were almost contiguous, but, near the periphery especially, were separated by loose connective tissue of the snail. It is clear that the sporocysts do not migrate and none was found in any other part of the snail. No free cercariae were found in the hemocoele.

The snail had been isolated for three weeks in the narrow confines of a finger bowl and during this time had liberated thousands of cercariae. Sections revealed hundreds of encysted metacercariae, located under the body wall and in the mantle, tentacles and edges of the foot. The cysts measured 0.1–0.12 mm. in diameter; the metacercariae were identical morphologically with the cercariae of *C. parvicaudata* and undoubtedly had been produced in this snail. It would appear, therefore, that infection with the asexual stages of the parasite does not confer immunity against subsequent invasion by cercariae.

The sporocysts (Fig. 7), which are undoubtedly second generation or daughter sporocysts, are oval to pyriform to clavate sacs, 0.3–0.9 mm. long and 0.15–0.35 mm. wide. There is a birth pore at one end of the sporocyst, visible only when a cercaria is emerging, and the cavity contains germinal cells, germ balls of various sizes and developing cercariae; sporocysts within sporocysts have not been observed. The germinal material, whether as single cells or as multicellular masses, always stains intensely and appears to be free in the cavity. In living sporocysts, the germinal elements are pushed about by movements of the young cercariae. The wall of the sporocyst consists of two layers: an inner layer of flattened cells in which the nuclei are clear and stain well, and an outer layer, the paletot, of large pigment bearing cells in which the nuclei are indistinct and do not stain. When the sporocyst is extended the lateral walls are thin and each layer is one cell in thickness, but if the sporocyst is contracted the cells may be compressed and superposed. At the ends of the sporocyst, especially the end where the birth pore is located, each layer of the wall may be several cells in thickness. The external ends of the cells of the paletot form a pebbled or beaded surface which is closely invested by a layer of flattened cells that are part of and continuous with the connective tissue of the snail. In the wall of the sporocyst there are scattered circular and longitudinal fibers, but living sporocysts have little or no motility when freed in sea-water.

The cercariae (Fig. 4) are oval to ovate from dorsoventral aspect and the wider portion may be either anterior or posterior to the middle of the body. Mature cercariae measure 0.14–0.36 mm. in length as the body is contracted or extended, and the width varies inversely with the length, measuring 0.05–0.12 mm. The body, but not the tail, is covered with minute, sharp-pointed spines, deeply embedded in the cuticula. The dorsal wall of the oral sucker bears a simple stylet (Fig. 5), 0.015 mm. long and 0.0032 mm. wide. There are small dorsal and ventral flanges on the stylet at the base of the conical tip. The tail is subterminal in attachment and is very active; it may be contracted to a length of 0.06 mm. or extended until it exceeds the length of the body. When the worm is attached or creeping or immobilized under a coverglass, the tail manifests nervous, twitchy movements. In swimming, the body is contracted into a short, wide form and bent ventrally while the tail is extended and lashes vigorously. The tail is almost circular in cross section and the terminal one-third is narrowed slightly. The larvae swim at random in all levels of the water and are more or less uniformly distributed in a finger bowl. They do not respond to light or move in any particular direction except upward; indeed, the swimming movements are weak and the lashing of the tail merely keeps them suspended in the water. Although they progress very little as a result of their own activity, they are carried about by currents in the water. An acetabulum is present and may protrude; it is median slightly posterior to the middle of the body, 0.03–0.05 mm. in diameter and is surrounded by a few small papillae and 68–72 spines. Under very slight pressure the sucker measures about 0.04 mm. in diameter, but becomes larger as the larva is flattened. The mouth opening is subterminal; the oral sucker is spherical to oval, 0.035–0.06 mm. in diameter and the dorsal lip bears 18 minute spines. The oral sucker is followed almost immediately by the pharynx which measures about 0.014 mm. long and 0.012 mm. wide and the esophagus extends about one-half the distance to the acetabulum. The digestive ceca end near the level of the caudal margin of the acetabulum.

The body is largely filled with glandular cells. There are six pairs of penetration glands, situated in the acetabular and preacetabular areas. These cells have a finely-granular cytoplasm which is colorless in transmitted light and stains a rose-pink with neutral red. They are irregularly flask-shaped, often lobed, 0.011–0.018 mm. in diameter, and the nuclei are 0.005–0.006 mm. in diameter. Ducts from these cells pass forward and open at the sides of the stylet. Under pressure, the contents of the glands stream forward at different rates and may cause enlargements of the ducts, especially at or near the oral sucker. Behind the nerve commissure, which spans the esophagus dorsal and immediately posterior to the pharynx, the body contains about one-hundred cystogenous cells. They are

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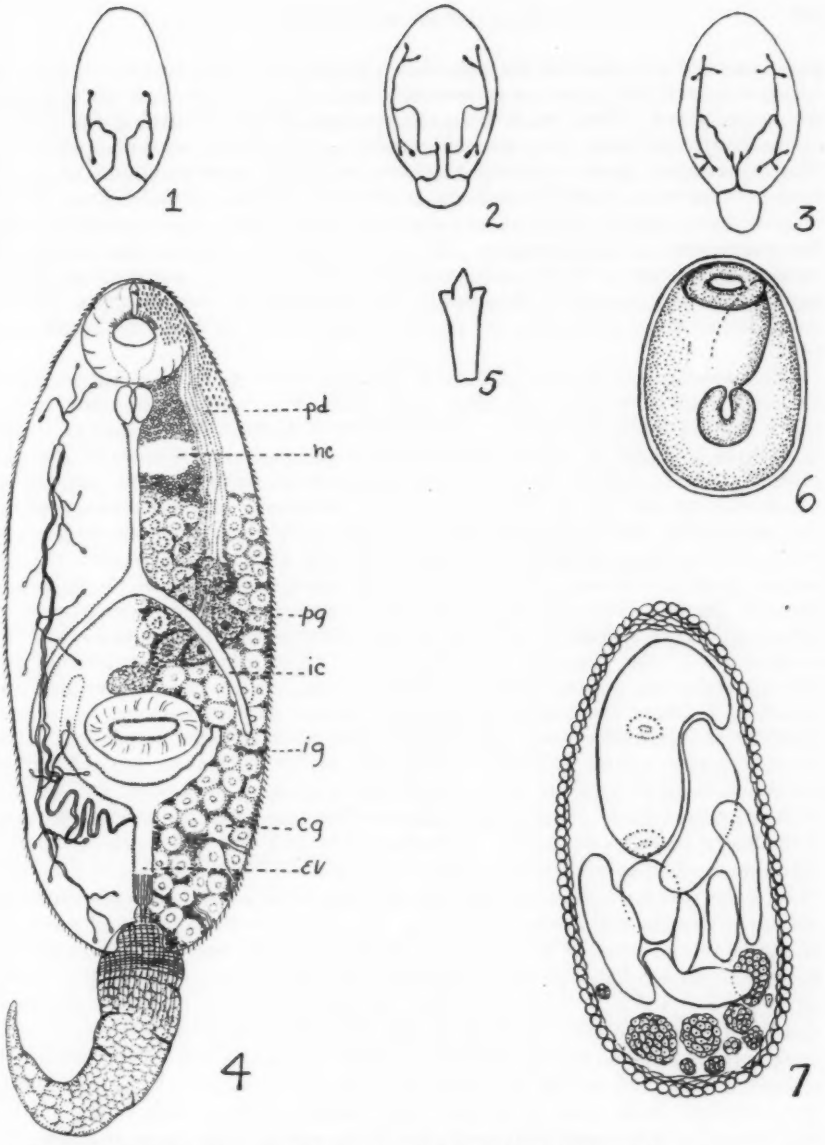
ABBREVIATIONS

cg—cystogenous gland
ev—excretory vesicle
ic—intestinal cecum
ig—intercellular granules
nc—nerve commissure
pd—penetration gland duct
pg—penetration gland



FIGURES 1, 2, 3. Development of excretory system in developing cercariae.
FIGURE 4. Mature cercaria, excretory system shown on the left, other structures on the right side.
FIGURE 5. Stylet, lateral view.
FIGURE 6. Metacercaria from *L. littorea*.
FIGURE 7. Sporocyst with developing cercariae.

about the size and shape of the penetration glands, but the cytoplasm is flaky in appearance, yellowish-green in transmitted light and reddish-brown when stained with neutral red. Short ducts from the cystogenous glands lead to the surface of the body and under pressure the contents are extruded as minute droplets. The intercellular spaces of the parenchyma are filled with thousands of small, refringent spherules, 0.0015–0.0025 mm. in diameter. When extruded in sea-water, these droplets adhere and coalesce; they are lighter than water and lie against the under side of the coverglass. They are soluble in alcohol, less soluble in xylol, and insoluble in N/10 acids or alkalies. They may be excretory products, unsaturated fatty materials, produced by the reduction of carbohydrates. For a discussion of these substances, the reader is referred to the paper by Stephenson (1947).

The development of the excretory system has been traced in young cercariae, removed from sporocysts. In large germ balls there are two excretory pores and a doubled excretory system. The number of flame cells increases as shown in Figures 1, 2 and 3. Later in development the posterior portions of the two collecting tubules fuse to form the median excretory vesicle and the constriction which cuts off the tail is situated immediately anterior to the excretory pores. Accordingly, in young cercariae there are two short ducts from the vesicle and two excretory pores; later the two ducts fuse and when the tail is shed, the excretory pore is terminal. It is to be noted, before the fusion of the collecting ducts of the two sides, that there may be a small anterior evagination on each collecting duct immediately anterior to the point where it later fuses with its counterpart of the opposite side, Figure 2. These evaginations produce the anterolateral arms of the future excretory vesicle. In the mature cercaria, the vesicle is Y-shaped and ventral in position; anterior to the pore there is a strong sphincter and the median stem extends more than half way to the acetabulum. The lateral branches extend forward on either side of the acetabulum, but the wall of the vesicle is weak, the shape is inconstant and varies with the amount and location of the contained fluid. The vesicle collapses when empty and the anterior portions of the lateral branches disappear. Collecting ducts, long and very much convoluted, open into the median stem of the vesicle a short distance behind the lateral branches. These ducts pass laterad and anteriad and near the middle of the body each bifurcates to form an anterior and a posterior branch. The pattern is the same in each quadrant of the body and consists of three sets of tubules, each receiving the capillaries from three flame cells as shown in Figure 4. The flame-cell formula of the mature cercaria is $2(3 + 3 + 3) + (3 + 3 + 3)$. In earlier literature, the idea is expressed that flame cells and capillaries divide to form the pattern of the mature cercaria, but there appears to be no critical evidence to support this idea and I have never seen a dividing flame cell or one in which there were two nuclei. It seems unlikely that functional flame cells divide and I am inclined to agree with Willey (1936) that they arise from undifferentiated cells in the parenchyma. It is probable that physiological conditions, perhaps the accumulation of excretory wastes, induce the formation of new flame cells from undifferentiated mesenchyme cells.

As mentioned above, the body of the cercaria is so filled with gland cells that other structures are obscured and the organs of later stages are relatively

undeveloped. A cluster of cells anterior to the acetabulum probably represents the rudiment of the copulatory organs and genital pore.

The cercariae may encyst in their sporocysts if left in sea-water; they will penetrate and encyst in excised parapodia of *Neanthes virens*, in *Littorina saxatilis*, *L. littorea* and *L. obtusata*, and in the small polyclad turbellarian, *Euplana gracilis*. In the turbellarian, the cysts were extruded after a few days. Newly formed cysts are spherical to oval, 0.1–0.15 mm. in diameter. The cyst wall is 0.002–0.0025 mm. thick, flexible and very tough. The cysts are not encapsulated by snail tissue and readily fall free when the snail is dissected. While the cysts are spherical in the host tissue, when released in sea-water they become ovate, with the body of the

TABLE I

	Cercaria roscovita	Cercaria parvicaudata
Host	*Littorina saxatilis nigrolineata*	*Littorina littorea, L. saxatilis, L. obtusata*
Sporocyst	Lemon colored, 0.7–1.5 mm. long, 0.25–0.45 mm. wide	Orange colored, 0.3–0.9 mm. long, 0.15–0.35 mm. wide
Cercaria	0.15–0.3 mm. long, 0.06–0.12 mm. wide	0.14–0.36 mm. long, 0.06–0.12 mm. wide
Tail	Subterminal, shorter than body, 0.08–0.3 mm. long	Same, 0.06–0.3 mm. long
Acetabulum	Postcentral, 0.034–0.05 mm. in diameter	Same
Stylet	Simple, 0.016–0.018 mm. long, 0.002–0.003 mm. wide	Simple, 0.015 mm. long, 0.0032 mm. wide
Cuticula	Sharp pointed spines	Same
Oral sucker	0.042–0.05 mm. in diameter	0.035–0.06 mm. in diameter
Pharynx	Near oral sucker, 0.014–0.018 mm. in diameter	Same, 0.012–0.014 mm. in diameter
Esophagus	Ends midway between suckers	Same
Penetration glands	Several, preacetabular, stain rose pink with neutral red	6 pairs, preacetabular, same staining reaction
Cystogenous glands	Numerous, secretion extruded under pressure	Same
Refractive globules	Very numerous in intercellular spaces	Same
Excretory vesicle	Y-shaped, collecting ducts enter stem of Y	Same
Flame-cell formula	$2(3 + 3 + 3) + (3 + 3 + 3)$	Same

metacercaria bent and the ends of the worm at the more pointed end of the cyst. On each side of the mouth of the larva, there is a row of 12–15 spines, larger than those regularly present in the cuticula.

DISCUSSION

The morphology of *C. parvicaudata* is strikingly similar to that of *C. roscovita* described by Stunkard (1932) from *Littorina saxatilis nigrolineata* taken at Roscoff, France. The two species are unique in that the collecting ducts of the excretory system open into the stem rather than into the lateral branches of the excretory vesicle. Comparison of the two species is facilitated by Table I.

Except for the difference in color of the sporocysts, the two species are almost identical. Differences in size may result from conditions under which the observations were made, *e. g.*, the amount of pressure to which the specimens were subjected at the time of measurement. The general structure of the two species, and especially the details of the excretory system, suggest that they are not only closely related but that they are probably members of the family Plagiorchiidae. Since members of this family are rare or unknown in marine fishes, and occur most frequently in birds and mammals, it is probable that some bird or mammal which frequents the sea-shore is the final host of *Cercaria parvicaudata*.

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